Synthesis of long-term impacts to pink salmon following the *Exxon Valdez* oil spill: persistence, toxicity, sensitivity, and controversy

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Executive Summary

Considerable controversy surrounds published studies on the short- and long-term effects of the *Exxon Valdez* oil spill (EVOS) on the pink salmon resources of Prince William Sound (PWS). The *Exxon Valdez* oil spill, the largest spill in United States history, occurred in a relatively pristine environment that was not compromised with other pollutants. Pink salmon were the most valuable fishery in PWS before the spill, the most studied species before and after the spill, and with their reproductive biology tied in large part to the intertidal "bathtub ring" of oil, they were the species in which long-term spill impacts were most likely detectable. The long-term impacts and the sensitivities to weathered oil found in a series of pink salmon studies are unprecedented and controversial. These studies will change the scientific view of long-term damage from oil spills and have significance far beyond understanding the long-term consequences of the spill to PWS. This review focuses on the major differences between studies funded by the Exxon Oil Company and Natural Resource Damage Assessment (NRDA) studies and, where possible, attempts to reconcile conflicting results.

Short-term Effects on Fry in the Marine Environment

Short-term damage to pink salmon fry in PWS was observed by NRDA researchers, but not by Exxon researchers. NRDA researchers concluded that fry entering and migrating through spill areas were exposed to oil in 1989, that growth rates of oiled fry were depressed, and that the population was reduced as a result of the spill. Exposure to oil was evident in both wild and hatchery fry; polynuclear aromatic hydrocarbon *(*PAH*)* concentrations in tissue were elevated, cytochrome P4501A was induced, and oil globules were observed in fry stomachs. The principal route of fry exposure was probably through ingestion of oil droplets rather than through waterborne exposure. In contrast, Exxon researchers found no evidence of any impact of oil on fry in 1989 and no evidence of population effects. Both Exxon and researchers for the Exxon Valdez Oil Spill Trustee Council agreed that some nearshore habitats remained contaminated a year after the spill; however, little oil was bioavailable to fry, and fry growth was not affected significantly in 1990 or later.

Field Studies: Long-term Impacts on Embryos in Oiled Streams

In contrast to the short-term damage observed in pink salmon fry, long-term damage to incubating embryos in PWS was observed by NRDA studies. Elevated embryo mortality in oiled streams continued through 1993, 4 years after the spill, and was an unprecedented finding. Continued mortalities in oiled streams, coupled with availability of oil in stream deltas, demonstrated significant and persistent effects of oil. Elevated embryo mortality in oiled streams through 1993, reproductive impairment in hatchery-incubated eggs from oiled streams, and the confirmation of embryo sensitivities to PAH by laboratory study were strong evidence of adverse long-term impacts on pink salmon. NRDA researchers hypothesized that high molecular weight PAH in weathered oil leaches from oiled stream banks into salmon redds, providing a mechanism for these long-term impacts. In contrast, Exxon researchers found no evidence of in-stream oil or increased embryo mortality. There is little possibility of reconciling two different experimental designs.

Laboratory Studies: Long-term Effects on Embryos Exposed to Weathered Oil

Laboratory studies designed to emulate post-spill conditions in PWS verified that embryos are sensitive to long-term exposures to weathered oil in the low part per billion (ppb) range of PAH. Mortalities, abnormalities, histopathological damage, and other biological effects increased with

embryo exposure to ppb concentrations of PAH. Delayed impacts on marine growth and survival were measured in returning adults exposed as embryos to ppb concentrations of weathered oil, further evidence that the embryo is vulnerable and sensitive. Sensitivity of salmon embryos to weathered crude oil at ppb concentrations is unprecedented, but parallel laboratory study also showed that Pacific herring (Clupea pallasi) embryos were similarly sensitive. These laboratory tests have been criticized, but not replicated, by Exxon researchers.

Significance Relative to Prince William Sound

Both NRDA and Exxon researchers conclude that long-term damage in the pink salmon population in PWS as a whole was not evident. The population collapse of 1992 and 1993 was significant in PWS, but direct linkage to oil toxicity is unlikely. Long-term oil impacts at the stream level were likely, but populations rebounded quickly, probably as a result of the short 2-year life cycle and the influence of strays.

Significance of Long-term Studies to Urban Estuaries

Lessons from these studies have broad implications concerning the relationship of urban estuaries to human-generated pollutants. Incubating embryos are sensitive to long-term exposure to low-level PAH concentrations because they sequester toxic hydrocarbons from low-level or intermittent exposures in lipid stores for long periods, and because developing embryos are highly susceptible to the toxic effects of pollutants. Weathered oil can be persistent, biologically available for a long period, and toxic to sensitive life stages. Urban runoff with low ppb concentrations of PAH can damage the reproduction of aquatic fauna. In the absence of further laboratory study with other fish species, the toxicity threshold of approximately 1 ppb aqueous PAH, derived from sensitive early-life stages of pink salmon and Pacific herring, should be used to estimate risk. We recommend that government standards for dissolved aromatics should be revised to reflect this threshold and adequately protect critical life stages and habitat.

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I. INTRODUCTION

The grounding of the T/V *Exxon Valdez* in Prince William Sound (PWS) on 24 March 1989 resulted in nearly 42 million liters (258,000 barrels) of crude oil being spilled, and the subsequent oiling of hundreds of kilometers of PWS shoreline; it was the largest oil spill in United States history. Damage attributed to the spilled oil was measured in several fish species, including pink salmon (*Oncorhynchus gorbuscha*) - the most valuable fishery in PWS before the spill and the most-studied species before and after the spill. Estimation of short and long-term damage of the spill to pink salmon differ greatly between researchers under contract to the United States government Exxon Valdez Oil Spill Trustee Council (hereafter scientists funded by the Council will be referred to as government researchers) and those under contract to Exxon Oil Company researchers, thus fueling a long-running dispute over the interpretation of the research. The controversy stems from three sectors of research, each with unprecedented findings: 1) long-term persistence of oil in spawning habitat, 2) elevated embryo mortalities in oiled spawning streams 4 years after the spill, and 3) the demonstration of sensitivities of embryos to part-per-billion (ppb) concentrations of the toxic fraction of crude oil.

The suite of pink salmon studies demonstrated long-term impacts of the oil spill, which are unprecedented, and they have altered our perception of oil-spill impacts. These studies are important to the understanding of long-term impacts of the oil spill on Prince William Sound and have, more importantly, implications for understanding the possible effects of non-point source pollution in urban estuaries. The *Exxon Valdez* oil spill started as a major point source pollution problem, but after 2 years, the pollution issue evolved to a non-point source event with long-term persistence and impacts. This has further fueled the controversy between industry and agency researchers. The general goal of this report is to summarize the findings of all the *Exxon Valdez* Oil Spill (EVOS) pink salmon studies, give the basic results from the two groups of studies, and comment on the conclusions of the two different groups of researchers.

A. PINK SALMON STUDIES ARE CONTROVERSIAL

The bulk of the studies of pink salmon following the EVOS fall into two categories; those contracted by Exxon, and those funded by the EVOS Trustee Council as part of either the Natural Resource Damage Assessment or the Restoration Programs. In some instances, these studies agree, but in several cases, especially the areas involving effects on various life stages, similar studies, but with different experimental designs, have led to differing findings and conclusions. Studies have examined both short and long-term effects, and disagree on the extent of the damage, and even to the existence of damage to pink salmon. Several studies examine the same issue but were seldom conducted identically. Consequently, most of the research effort is more complimentary and open to differences in interpretation. It is our goal to: 1) review and present the information in a way that will permit the reader to form an opinion as to the real, if any, effects of the EVOS on Prince William Sound pink salmon, and 2) call the reader's attention to the potential for damage from low-level, non-point source pollution of urban estuaries.

B. IMPORTANCE OF PWS PINK SALMON

Returns of wild pink salmon to PWS have ranged from 1.8 to 21.0 million since 1978 (Bue et al., 1996). Wild pink salmon are a major component of the PWS ecosystem and, with hatchery pink salmon, are the dominant contributor to the region's commercial fisheries (Figure 1). In 1989,1990, and 1991 wild pink salmon contributed 24%, 29%, and 19% respectively to the total PWS pink salmon catch; total catch ranged from 24.5 to 44.9 million fish (Sharr et al., 1995). Due to the importance of PWS wild pink salmon, many studies were conducted following the EVOS. Studies were conducted to assess the impact of the spill on pink salmon life cycle stages, as well as to assess effect at the population level.

C. PINK SALMON LIFE CYCLE

Due to their life history cycle (Figure 2), pink salmon were considered to be especially at risk from the oil spill for two reasons: (1) pink salmon spawn in the intertidal reaches of streams, including the area known as the "bathtub ring," where the heaviest strandings of oil occurred, and (2) pink salmon emerge from the spawning gravels and migrate immediately to the potentially oiled estuaries for their initial feeding and growth. Approximately 75% of PWS pink salmon are intertidal spawners (Helle et al., 1964), spawning in freshwater reaches of streams at low tide. Spawning occurs from mid-July through September. Eggs deposited in the intertidal gravels hatch between late October and December, and alevins continue to incubate in gravel until about April. Following emergence, fry move immediately into the marine environment, where they feed initially in nearshore waters before they migrate to open ocean. Unlike other Pacific salmon, the life cycle of pink salmon is two years, with approximately 14-15 months in seawater. Hence, there is an even-year stock and an odd-year stock for each stream.

D. OIL IN THE ENVIRONMENT OF PINK SALMON

Oil in the intertidal pink salmon spawning habitat was not anticipated at the time of the spill; the oil exposure issue is complex and has several parts, each of which has led to a series of misunderstandings. First, many researchers did not perceive that stranded oil along the shoreline could impact streams with intertidal spawning habitat. Oil floats, so little or no effort was exerted in the years immediately after the spill to define oil persistence and availability to pink salmon embryos and alevins in the gravel of freshwater spawning streams. Second, before the oil spill, oil toxicity studies focused on short-term tests with 1- and 2-ringed aromatic hydrocarbons. Larger 3- and 4-ringed compounds were known to be more toxic, but they were also less available and less damaging in short-term tests. Historically there was a reliance on

short-term acute toxicity literature from the 1970s to define and predict the sensitive life stages and the toxic fractions of concern; long-term exposures were not believed to be a significant problem following spills. Consequently, most studies following the spill were designed to focus on short-term toxicity issues.

E. LIFE CYCLE STAGES POTENTIALLY EXPOSED TO EXXON VALDEZ OIL

The EVOS occurred in late March, before fry emerged from PWS stream bed gravel. Hatched 1988-brood larvae with a little yolk, called alevins, were near the end of their development in the spawning redds of streams. Within several weeks of the spill, they finished consuming their yolks, emerged from the stream gravels, and migrated immediately into nearshore estuaries. First feeding and early growth was near shore, and within a month, fry moved offshore and continued their migration through the sound to the open ocean. Exposures could have occurred to alevins in the streams, or to fry early during their stay in the near shore environment, and decreased thereafter.

For 1989 brood, potential exposure began when the returning adults appeared in the Sound in July. Adults spawned in August or September, and the early embryonic stages were potentially exposed to intermittent doses of oil for a long time. Residual oil decreased in succeeding years, and exposures would presumably be less in succeeding brood years. Short-term effects on fry and growth were most likely from the 1988-brood fish emerging in 1989 during the peak of the spill, whereas long-term effects from chronic exposure were most likely to the 1989-brood fish, and possibly in later brood years.

F. SEQUENCE OF REVIEW

In order to better enable the reader to compare conflicting methodologies, data, results, and

conclusions, we separate our presentation into the following areas of discussion: (1) short-term effects on fry growth and mortality during marine exposure in 1989 and 1990, (2) long-term effects on embryos and alevin development from residual oil in stream deltas, (3) laboratory tests on sensitivities of embryos, (4) the significance of these studies to PWS, and (5) the significance to urban estuaries. In the review of short and long-term effects, the availability of oil will be presented for those life stages impacted, along with the perspectives of both Exxon and government researchers.

II. SHORT-TERM EFFECTS ON MARINE SALMON FRY

At the time of the spill, fry were beginning to emerge from the spawning gravels, feed along the shoreline, and out-migrate through the spill area. There were two immediate concerns: (1) fry were reportedly the most sensitive life stage to short-term acute oil exposures, particularly when in seawater (Moles et al.,1979), and (2) the threat from an acute exposure was considered significant, given the volume of spilled oil in the migration pathway, particularly in north facing bays. In addition to direct effects on fry from the oil were the concerns that growth of fry in the early weeks could be affected. This was a considerable concern because it is often the success, or the lack of success, in the first few weeks of marine survival that determines the success of a year class in pink salmon (Parker, 1971, Heard, 1991; Mortensen et al., 2000). Interruptions in early feeding affect size and growth; small fish are targeted by predators with a net decrease in populations. Damage assessment studies focused on the threat from exposure directly to oiled water, growth of hatchery and wild fry, contamination, and the subsequent effects on the returning populations after the spill.

A. DEPOSITION AND AVAILABILITY OF OIL TO PINK SALMON FRY

The rupture of the T/V *Exxon Valdez* hull occurred under calm sea conditions in PWS. Nearly 42 million liters of crude oil was carried slowly with the prevailing currents in a southwesterly direction. Within 3 days, a strong northerly wind moved the oil rapidly through the southwest passages of the sound toward the northwestern Gulf of Alaska. Hundreds of kilometers of beaches in southwestern PWS were contaminated with *Exxon Valdez* oil (EVO). An estimated 20% of the spilled oil evaporated, and 40% of the oil mass coated the intertidal areas of PWS (Wolfe et al., 1994); some of these areas surrounded or were in the near vicinity of, pink salmon spawning streams. Approximately 31% of identified PWS pink salmon spawning streams in the

southwest district were oiled to some extent (Geiger et al., 1996). About 14% of the EVO was recovered by cleanup operations; however, by the fall of 1992 an estimated 2% of the spilled oil mass (800,000 liters of oil) remained on the beaches of PWS (Wolfe et al., 1994; Spies et al., 1996). Except for relatively unweathered oil found protected by overlying mussel beds or beneath the surface on large cobble or boulder beaches (Babcock et al., 1996; Harris et al., 1996; Brodersen et al., 1999), the bulk of the remaining oil was somewhat weathered. Surface exposed oil continued to weather significantly with time; subsurface oil weathering was variable but much slower than exposed oil.

The spread of the spilled oil in PWS in 1989 resulted in the potential for exposure of pink salmon life stages. Oil in the water column, or present as small particulates, and oil-contaminated food was available to contaminate pink salmon fry during their early marine migration. This threat to a potentially acute exposure was the primary concern of researchers for pink salmon during the first two months of the spill.

(1) Oil In The Water Column: Acutely toxic concentrations of oil in the water column were not found in the first month following the spill, much to the surprise of many researchers that saw the magnitude of the spill and were familiar with the toxicity levels reported in the literature of the 1970s and 1980s. Short and Harris (1993,1996b) found that low concentrations of polynuclear aromatic hydrocarbons (PAH) at shallow depths (1 and 5 m) were pervasive within the slick trajectory in the first 6 weeks of the spill; PAH composition was similar to EVO. Concentrations of PAH in water sampled adjacent to heavily oiled beaches ranged from 1.26 to 6.24 ppb and were usually higher at 1 m than 5 m depth, but not always. Neff and Stubblefield (1993) found surface PAH concentrations as high as 30 ppb, although most of their measurements were deeper and later than Short and Harris, and most of their measured concentrations of PAH were lower than the Short and Harris measurements. The 96-h LC_{50} of WSF for pink salmon range from 1,500 to 8,000 ppb depending on life stage, salinity, and type of

exposure (Rice et al., 1977, 1979; Moles et al., 1979; Korn and Rice, 1981). All measured PAH concentrations were well below concentrations found to be acutely toxic during 28-d exposures of marine fauna to water-soluble fractions (WSF) (Moles, 1998).

When comparing oil studies, it is essential that the composition of the oil fraction in the study be known, as well as the concentration. The lab studies of the 1970s and 1980s typically used a WSF composed primarily of 1-2 ringed aromatic hydrocarbons. In the EVOS measurements, both Short and Harris (1993, 1996b) and Neff and Stubblefield (1993) measured PAH in the water column, not WSF; hence, the composition was different from that reported in WSF studies. Concentrations of PAH cannot, and should not, be compared to WSF LC_{50}s, because the 3- and 4-ringed compounds are 10-1000 times more toxic than the 1-2 ringed aromatic compounds in WSF (Black et al., 1983). Most of the literature before the spill indicated short-term acute effects of WSF as the most likely route of exposure to fish because of their relative solubilities in water and thus, their availability to organisms.

Because of the mixing energy supplied by 70-knot winds about day 3-4, many were surprised that the aqueous oil concentrations after the spill were not higher. Winds had several effects: they spread the oil rapidly into a much thinner layer and mixed some oil into the water column, but they also increased the rates of removal of the more volatile light-end aromatic hydrocarbons. By the time the oil in water was first measured, researchers began to appreciate that this spill was not going to be an acutely toxic situation for the fauna below the surface of the water. Dead fish were not appearing, and oil concentrations in the water column were subacute.

Oil was available to fish. PAH persisted in waters adjacent to heavily oiled beaches for long periods (Short and Harris, 1996a), although there was a general decrease in water PAH with time (Short and Harris, 1996a; Neff and Stubblefield ,1993). North-facing beaches trapped the oil, apparently serving as reservoirs of PAH which continued to re-contaminate nearshore water. Elevated PAHs in the water column were still detectable in May 1989 and profiles were still matching well with EVO, indicating the oil was still relatively unweathered, except for the loss

of the 1 ringed aromatics (Short and Harris, 1996a) .

The nearshore environment was contaminated heavily by stranded oil, creating concern for the newly emerged fry, which use the nearshore habitat for shelter and for their first feeding. At high tide, the stranded oil was covered by shallow water and in close proximity to the fish and their prey. Contamination of the food supply was not evaluated, but there was concern that the fry might consume oiled prey or even mistake oil droplets in water as food, particularly near the shore of high-energy beaches.

The best evidence of oil availability below the surface was in caged mussel studies. Short and Harris (1996b) deployed clean mussels at several sites in PWS during the summer of 1989, and found oil contamination in the tissues. These mussels were deployed in cages below the surface, and were never exposed to oil floating on the surface. The mussels accumulated hydrocarbons, to concentrations of 50,000 ppb PAH, indicating frequent exposure, even if intermittent. Further, the composition of the oil in the mussel tissue was matched to EVO, indicating that the oil was not a WSF, but acquired in particulate forms.

(2) Biological evidence of oil exposure to pink salmon fry (1989/1990): In 1989, oil globules were found in fry stomachs, PAH were found in tissues, and P450 enzymes were induced. For these measurements, fry were collected in four oiled and four non-oiled areas from April through June 1989. Sturdevant et al. (1996) report the visual observation of oil globules in the stomachs of pink salmon fry captured at oiled sites in 1989. Concentrations of PAH in fry tissues (Figure 3) from the four oiled areas were significantly elevated above the concentrations in reference fry (Carls et al., 1996a). Likewise, cytochrome P4501A enzyme levels were significant in fry from the oiled areas (Figure 3). Cytochrome P4501A enzymes are known to be induced and elevated in vertebrates exposed to PAH, and are often used as a biomarker to indicate recent PAH exposure history. Tissue PAH and induction of P4501A were both significantly greater in fry collected from oiled areas in 1989 than in fry from reference areas of PWS. Composition of

PAH in fry tissues were similar to PAH in EVO, indicating ingestion of oil or oiled prey, rather than absorption from WSF (which would not have matched the EVO fingerprint). In a separate study, Willette (1996) reported that cytochrome P4501A induction also occurred in hatchery-released pink salmon fry, captured within a few weeks of release in oiled areas in 1989. In 1990, pink salmon fry exposure to oil was reduced markedly.

Carls et al. (1996a) collected four sets of fry samples from the same eight areas between April and June 1990. Unlike the 1989 fry samples, no significant differences in tissue PAH or P4501A induction were noted in the fry collected from oiled and un-oiled areas in 1990.

B. FRY MORTALITY AND GROWTH IN SALTWATER: 1989 AND 1990

Oil in the marine environment can affect salmon fry in a variety of ways. Oil WSF can be acutely toxic in the short-term (Rice et al., 1975, 1984); it can affect metabolism, reduce growth (Rice et al., 1975), and damage olfactory lamellar surfaces (Babcock, 1985). Reduced growth of salmon fry has been noted due to direct exposure to WSF (Moles and Rice, 1983), contamination of prey by WSF (Schwartz, 1985), and by direct ingestion of crude oil (Carls et al., 1996b). Due to the known effects of oil on salmon fry, and the emergence and migration of pink salmon fry at the approximate time of the EVOS, several studies were initiated immediately after the spill to monitor the consequences of the EVO to pink salmon fry.

(1) Fry mortality: Direct fry mortality was not reported by any of the scientists studying the effects of the EVOS on pink salmon. This is not surprising, for the water concentrations in the first few weeks were much less than the lethal concentration reported for acute, short-term exposures. Pink salmon fry are also reported to move rapidly from protected bays to migration corridors (Wertheimer and Celewycz, 1996), and if there were mortalities, they would be difficult to observe. Predation on moribund fry would be expected to be high, also making it improbable

to find direct mortalities, even if they did occur.

(2) Growth Of Pink Salmon Fry: Several studies were conducted, beginning shortly after the spill, to monitor the effect of the oil on the growth rate of pink salmon fry in seawater. Growth rates of pink salmon fry are typically fast in the first few weeks after emergence; this is a critical time that can have a large impact on population numbers. Fry are heavily preyed upon and depend on fast growth rates to grow out of the predator-susceptable fish sizes (Parker, 1971, Heard , 1991; Mortensen et al., 2000). Growth reductions reported in 1989 are debated by Exxon and government researchers. In1990, early marine growth of fry was not affected by the low concentrations of oil which remained a year after the spill (Wertheimer and Celewycz, 1996).

Brannon et al. (1995) monitored marine fry growth in two oiled areas over a 15-d period in May-June 1989. However, they did not compare growth of fry from contaminated bays with fry from reference areas, but with expected growth rates (Figure 4). On the basis of calculated fry growth, they concluded that fry growth in oiled areas was normal and would have been predicted on the basis of temperature and date. These growth rates indicate there was no catastrophic effect, but without measurements of growth in non-oiled fry from non-oiled areas, the study lacks the ability to state there was no oil effect on growth. Government researchers (Wertheimer and Celewycz, 1996), on the other hand, measured growth of fry captured in both oiled and non-oiled areas of PWS; they did not depend on calculated projections of growth.

Wertheimer and Celewycz (1996) reported less growth of pink salmon wild fry recovered from nearshore waters in oiled areas between 10 April and 26 June 1989 than in fry from reference areas (Figure 4). No difference was noted in fry growth when fry were captured in bays, but differences were noted in fry captured in oiled migration corridors in 1989 compared to non-oiled corridors. It was suggested that fry spend little time in protected bays before they move into migration corridors, consequently, there is little opportunity to detect differences in fry growth between oiled and reference bays. Wertheimer and Celewycz (1996) also report that pink

salmon fry were more abundant in non-oiled areas than in oiled areas in both 1989 and 1990, but they attribute the difference to geographic differences in fry production and migration.

Willette (1996) measured decreased growth in hatchery pink salmon fry collected in oiled areas than in fry captured in un-oiled areas, even though all the fry were released from hatcheries at known dates and tagged with coded-wire tags (CWT) (Figure 4). Fry were released from the hatchery in May and June 1989 and sampled from the third week of May until the beginning of July. Tagged fry recovered in 1989 from moderately oiled areas were significantly smaller than tagged fry, of the same hatchery groups, recovered from non-oiled or lightly oiled areas. Depressed fry growth in 1989 coincided closely with significantly greater induction of cytochrome P4501A in these fry. Unlike the wild fry, release dates and sizes of the hatchery fry are known, and growth rates can be measured accurately; there can be little doubt of an effect of oil on growth rates.

Government researchers report PAH exposure to wild pink salmon fry in 1989 on the basis of P4501A induction, tissue hydrocarbon concentrations, and oil droplets seen in fry stomachs and intestines. Reduced growth of exposed wild fry in 1989 is attributed to the oil exposures. Exxon researchers, on the other hand, report no effect on the growth of fry taken from oiled areas. Studies overlapped somewhat in sampling time and sampling area, yet investigators report different results. Sampling gear was different for the two groups thus, slightly different habitats were sampled, and although sampling times did overlap, they were not identical. The major difference between the growth studies in 1989, however, was the lack of controls in the Exxon study by Brannon et al. (1995). Industry researchers did not sample fish from non-oiled areas, and their determination of growth was based solely on calculated values.

In 1990, no government or Exxon study found growth rate differences. In 1990, Brannon et al. (1995) modified their study design, and sampled four oiled bays and four reference bays for fry growth. They concluded there were no differences in the growth rate of the groups. All sampled fry were captured with a surface trawl within bays. Wertheimer and Celewycz (1996)

also report no effect on growth of marine fry in 1990. They collected fry from nearshore waters in 2 bays and from 2 migration corridors of both oiled and reference areas, April-June 1990. No differences in growth were noted in fry from either bays or migration corridors in 1990. Growth rates of hatchery-released fry captured in moderately oiled areas in 1990 were not significantly lower, but were marginally lower in 1991 (Willette, 1996). In 1990, biological evidence of oil contamination in fry was not found.

The mechanism of decreased growth is probably related to energy expenditures needed to deal with the toxic exposure. In lab tests, decreased growth in pink salmon fry occurs at 33% of the LC_{50} (about 0.4 ppm) after 40 days of exposure to WSF (Moles and Rice, 1983). The higher the concentration of oil, the greater the observed reduction in growth. Juvenile pink salmon exposed to nearly lethal levels of WSF do not grow (Moles and Rice, 1983). Carls et al. (1996b) found significant reductions in pink salmon fry growth when exposure was via food contaminated with North slope crude oil. Significant reductions were reported when oil concentrations were as low as 1.3% of the LC_{50}. This is a lower percent of the LC_{50} than reported by Moles and Rice, but Carls et al. used a more toxic PAH fraction that included 3- and 4-ring PAH. The concentration of WSF that affects growth in pink salmon is the same concentration that increases respiration rates (Thomas and Rice, 1979, Moles and Rice, 1983), suggesting energy for growth is diverted to hydrocarbon metabolism and excretion. PAH effects on growth have been measured in other salmonids as well (Moles et al., 1981, Vignier et al., 1992).

Food availability or consumption was not interrupted (Sturdevant et al., 1996) and was not related to the decrease in pink salmon fry growth in 1989. Fry captured in oiled areas consumed as much food as those taken from reference areas. Oil globules or oil sheen were observed in stomachs of fry captured at oiled sites. The observation of oil associated with the stomachs of fry in oiled areas, but not reference area fry, suggests that ingested oil is a route of hydrocarbon contamination. In laboratory studies, WSF and crude oil-contaminated food reduced growth and feeding at high doses (Schwartz, 1985; Carls et al., 1996b). However, at lower doses,

consumption of oil-contaminated food reduced growth without lowering food consumption in both pink salmon and Atlantic salmon *Salmo salar* by reducing food-conversion efficiency (Vignier et al., 1992, Carls et al., 1996b). Rice et al. (1977) suggested that energy demands imposed due to PAH metabolism may remain elevated during the period of hydrocarbon depuration. It is thus probable that ingestion of oil from contaminated environments in PWS resulted in decreased growth rate without a decease in food consumption.

(3) Growth And Tissue PAH Loads: Decreased growth of fish exposed to oil has been demonstrated in several studies, but the comparison of tissue loads in the lab and field tests by Carls et al. (1996a, b) indicate that the field exposures in oiled areas of PWS in 1989 were likely to have had significant effects on growth (Figure 5). Pink salmon fry fed low and moderate levels of oil had decreased growth (Carls et al., 1996b), similar to the decreases in growth of fry from oiled areas of PWS (Wertheimer and Celewycz, 1996). Further, the lab exposed fry had tissue PAH levels similar to fry collected in oiled areas of PWS in 1989 (Carls et al., 1996a). These observations are strong support for an oil caused effect on growth in wild fry in PWS in 1989.

Exxon researchers argue for no oil effect on fry in 1990 because fry condition was good (Brannon et al., 1995). Government researchers do not argue for an oil effect in 1990, but use of condition factor for support of an effect is probably not appropriate. Condition factor (ratio of weight to length) is sometimes used as a diagnostic measurement when assessing fish health during development, but it is of questionable value when used to assess toxic effects. Carls et al. (1996b) found pink salmon juveniles that ingested low and moderate dosages of crude oil-contaminated food had similar or higher condition factors than control fish, even though growth was reduced at those dosages (Figure 5). In PWS, growth of pink salmon fry was reduced in 1989 (Wertheimer and Celewycz, 1996), but condition factor was higher in 1989 and 1990 in fry from the oiled than from the non-oiled areas. Condition factor is not a sensitive measure of toxic

effects in lab or field tests with oil.

(4) Significance of growth effects at the population level: Geiger, et al. (1996) estimate that 1.9 million wild adult pink salmon did not return to PWS in 1990, due primarily to increased predation caused by a lack of growth of fry in the nearshore environment in spring of 1989. They estimate that this loss accounts for approximately 28% of the potential wild-stock production from the southwestern part of the Sound returning in 1990. However, the total regional returns to all of PWS (hatchery plus wild, and all districts within the sound) were high in 1990, and the excellent marine survival of all fish tends to obscure the estimated losses in fish from the southwestern district of PWS. Maki et al. (1995) point to the high levels of return to all of PWS and conclude that the oil spill had no effect at the population level, probably a true statement for the entire sound, but probably not at the stream level. Although high population levels following the spill indicates that there was no massive catastrophic effect, they are poor evidence and too simplistic an argument for or against an oil effect. This line of reasoning fails to account for the influence for interannual variation (from differing marine survival rates, hatchery production and straying, differential removals from predation or fishing, etc.), while combining stocks that were in the spill area with those that were in the eastern part of the sound and outside the immediate spill area. Only about a quarter of the wild pink salmon population was located in the spill area where significant effects were more likely.

Neither Geiger et al. nor Maki et al. measured population impacts at the stream level, where oil effects were likely to occur in many streams from the southwestern district, where about 31% of the streams were oiled (Geiger et al., 1996). Measuring population impacts at individual streams is problematic. There are too many streams in PWS to manage the fisheries on an individual stream basis hence, there was no way to estimate the impacts at the individual stream. This problem is exacerbated further by straying, which was found to be considerable following the spill. Pink salmon straying levels, whether caused by oil in the environment or natural, were

measured in several streams 1989-91 (Sharr et al., 1995). Straying by hatchery and wild fish was greater than anticipated, and when straying rates are as high as 50%, they can mask poor returns of adults originating from that stream. In an extensive and statistically rigorous study, Wertheimer et al. (2000) found no evidence that these high rates of straying were attributable to oil exposure of incubating salmon eggs.

The estimates by Geiger et al. (1996) of non-returning fish, based on poor fry growth in 1989, are probably valid. The observations by Maki et al. (1995) that there were no regional population impacts cannot be proved, because the region consists of hundreds of non-impacted streams intermixed with oiled streams and interannual variation was not taken into account. Any impacts at the individual stream level were masked by the general decadal trend in high survival rates of fry and by the high rates of straying by hatchery and wild fish.

C. RECONCILIATION OF DIFFERING GROWTH RESULTS

Exxon researchers report no effect of the EVOS on pink salmon growth, whereas government researchers report significant growth effects. Certainly there was no catastrophic effect on growth and early mortality, but the more subtle effects on growth would require rigorous designs to detect effects in 1989. Government and Exxon designs differed significantly and form the basis of interpretation differences between the two research groups.

(1) Design: First, Brannon et al. (1995) sampled only contaminated areas in 1989, and compared the observed growth rates to calculated expected values, rather than to controls in non-oiled areas. This would be sufficient to detect catastrophic effects on growth, but not subtle differences between oiled and un-oiled areas, nor take into account variance associated with interannual differences. This contrasts sharply with the studies by Wertheimer and Celewycz (1996) and of Willete (1996), where growth of fry from oiled and un-oiled areas were compared

and found to differ. Wertheimer and Celewycz measured apparent growth rates by measuring the mean size in a given environment over time that mean may have been affected by immigration of later emerging fry, but the means changed more slowly in the oiled environments. Willette (1996) found results similar to those of Wertheimer and Celewycz while comparing growth rates of CWT hatchery fry of known size and age and collected in oiled and unoiled areas. By 1990, Brannon et al. (1995) had modified their design, and like the government researchers, found no effect on growth after the first winter, when stranded oil along the shorelines had been reduced greatly.

Second, the sampling designs of the two groups were slightly different and may play a role in the differences. Wertheimer and Celewycz (1996) sampled fry nearshore in both oiled and non-oiled areas in 1989 and 1990. Brannon et al. (1995) sampled fry offshore, where the fish are slightly larger. Studies conducted before EVOS (see review by Heard, 1991) revealed that pink salmon fry need to reach a critical size threshold before moving offshore. Brannon et al. (1995) sampled fry offshore, thereby sampling fry that met a specific size criterion. They would be unlikely to sample fish in the offshore environment with poor growth rates, for these fry would not have reached the critical minimal size for offshore migration. The fry sampled by Wertheimer and Celewycz (1996) from the nearshore environment had not yet reached appropriate size for offshore migration.

(2) Exxon Researchers Argue For No Population Effect From Decreased Fry Growth:

Exxon researchers point to the high salmon runs of 1990 through 1991 and argue for no population effect of the EVOS on pink salmon (Maki et al., 1995). On the other hand, the model suggested by Geiger et al. (1996) predicts significant population effects. Certainly marine survivals were outstanding in the region, and in the rest of the state, and these excellent marine survivals tend to mask the impacts of the spill. Further, only a fraction of the total area was impacted by the spill, probably less than one fourth of the PWS production was exposed as fry in

the first few weeks of the spill; hence, the impacted fish were diluted with substantial numbers of fish from the PWS region with little or no exposure. Maki et al. also report that no correlation exists between escapement densities and PAH concentrations in the spawning streams.

Straying studies indicate that hatchery strays can account for up to 50% (Sharr et al., 1995) and up to 97% (Joyce and Evans, 1999) of the escapement to individual streams; hence, their survival could easily mask the wild escapement to a particular stream and preclude any measurement of an oil effect. As with any environmental experiment, there are multiple variables, and precise estimates of missing fish is a complicated and clouded issue, but the weight of the evidence indicates that fewer fish returned to oiled streams even though there were high returns to the region.

D. CONCLUSIONS

Fry entering and migrating through oiled areas were exposed to oil and apparently suffered decreases in growth rate, and populations on a local level were affected. Wild and hatchery pink salmon fry were exposed to EVO in 1989 as evidenced by tissue PAH concentrations and induction of P4501A (Carls et al., 1996a; Willette, 1996), and oil globules observed in the stomachs of fry (Sturdevant, et al., 1996). The primary route of exposure appears to have been through ingestion, as supported by the oil globules observed in the gut, and by the composition of oil in the tissues having the fingerprint of EVO, which could not occur with merely a WSF. Further, the concentrations of oil in the water column were low and declined with time. The nearshore habitat was contaminated, was often high in energy (wave action), and the availability of oil droplets or contaminated food was considerable in 1989. The EVO fingerprint in contaminated fry was the same as the EVO fingerprint in mussels caged below the surface-indicating the availability of EVO below the surface, and the source of contamination as "droplets" rather than a WSF. The lack of an effect on growth in 1990 further supports ingestion

as the primary route.

Although some nearshore habitats were contaminated in 1990, the availability of stranded oil on the surface of the beach was minimal, not available for widespread wave dispersion (after the first winter), and consequently droplets of oil were not available at the time of the emigration in spring 1990. The energy cost in dealing with ingested oil explains a reduction in growth in 1989 and drives the model to predict a population reduction caused by reduced survival through increased predation. The high population return for the region, which include most streams that were not affected, does not outweigh the exposure data and the two studies that measured impacts on growth that would have subsequently led to decreased survival for the exposed fish.

III. FIELD STUDIES; LONG-TERM IMPACTS ON EMBRYOS

Long-term effects would be significant when judging the impacts of a spill, but they are normally difficult if not impossible to measure. Liters spilled and numbers of oiled carcasses found in the first few weeks following a spill are the traditional measures of assessment. Lingering contamination is sometimes monitored, but long-term damage is seldom measured for three reasons: (1) population numbers are seldom known before and after a spill, nor the natural variance of the population, (2) the lingering oil is often in an environment contaminated with a suite of other pollutants, and long-term biological damage from the spilled oil would be difficult to separate from other ongoing damages, and (3) monies are rarely, if ever, available over years that such an evaluation requires.

The spill in PWS was unique: pink salmon numbers and biology were well understood before the spill, and other pollutants were virtually absent compared to the oil from the spill and to hydrocarbons present in urban areas. Separating oil spill damage from natural fluctuations was more feasible in this spill than in any other in history. Hence, considerable effort was devoted by both government and Exxon researchers to define the significance of long-term damage, if any.

The evidence in recent years points toward a problem of persistence of oil in spawning habitats and is related to two primary issues: (1) toxicity of the weathered oil (and a change in our understanding of the toxic fraction of oil), and (2) sensitivity of embryos to long-term exposures (and a change in our understanding of sensitivity based on acute LC_{50}s of the past). We will examine the evidence of long-term exposure and long-term damage (including embryo mortalities in outlying years, reproductive impairment, and delayed impacts). The issue of toxicity (weathered oil) coupled with sensitivity of embryos will be discussed, because these two concepts are critical to interpretation of long-term damage.

A. OIL PERSISTENCE, AVAILABILITY TO PINK SALMON EMBRYOS, AND WEATHERING

There is considerable disagreement between Exxon and government researchers on the long-term effects to pink salmon eggs, embryos, and alevins. Disagreement begins with the issue of exposure. How do these organisms incubating in the gravel of freshwater streams become contaminated with oil, if oil floats? The controversy is fueled because measurements taken in 1989-90 were based on some shaky assumptions, and researchers did not anticipate long-term exposure or that damage would occur. Eggs deposited in the summer and fall of 1989 would produce the first embryos to be potentially exposed to EVO, and the first embryos subject to long-term exposure to oil for up to 8 months of incubation in the stream gravels. Exposure could occur from contact with oiled spawning gravel or by exposure to PAH-laden water.

(1) Oil Persistence In Intertidal Stream Sediments: Exxon researchers found little oil in streambed sediments from 1989 to 1991. Brannon et al. (1995) analyzed surface sediment from 8 to 11 reference stream beds and 8 or 9 oiled streambeds in 1989, 1990, and 1991. Streams were classified as oiled or reference on the basis of visual observation of oil in 1989. In oiled streams, mean PAH sediment concentrations ranged up to 267 ppb in 1989, up to 2818 ppb in 1990, and 236 ppb in 1991. In-flowing fresh water would restrict the direct contact of oil with streambed sediments, and thus stream bed surface sediments would be expected to have low, if any, hydrocarbon loads.

In contrast to measurements of oil in stream sediments, government researchers examined the contamination of stream deltas, in 1989-91 and again in 1995. Murphy et al. (1999) evaluated the intertidal area beside streams as a source of PAH for spawning gravels; no sample was taken from within a stream. Murphy et al. determined total hydrocarbon and total PAH in sediment samples taken in 1989, 1990, 1991, and 1995 from the delta (above or adjacent to the

stream bed) of PWS streams. A map of one stream delta (Figure 6) demonstrates the distribution of oil in 1989 and 1995 along the sides of a stream. The Alaska Department of Fish and Game sampled the stream deltas: 172 in 1989, 12 in 1990, and 4 in 1991. Twelve of the same streams were sampled intensely in 1995. In 1989–1991 samples, PAH concentrations in subsurface sediment samples from 44 stream deltas ranged from 0 to 968,000 ppb. At nine of the same streams sampled by Brannon et al. (1995), Murphy et al. (1999) report a range of PAH in 1989 of 0 to 311,000 ppb in the stream delta (Table 1) . This compares to a range of 1-2,818 ppb PAH in the stream bed surface sediment of these nine streams. In the 12 stream deltas re-sampled in 1995 by Murphy et al. (1999), PAH sediment concentration averaged 2- 2,800 ppb, values equivalent to stream bed surface sediment assayed by Brannon et al., 4 - 6 years earlier.

Table 1.--Total polynuclear aromatic hydrocarbon concentration (ppb) in samples taken from the same 9 streams in Prince William Sound, Alaska, by two sets of researchers: samples from surface of the wetted streambed by Brannon et al. (1995) and samples from the stream delta by Murphy et al. (1999).

	Streambed surface[b]				Stream
	Fall	Spring	Fall	Spring	delta[c]
Stream[a]	1989	1990	1990	1991	1989
16180	194	182	2	5	1,316
16280	1	384	21	224	24,491
16630	233		31	96	26,969
16650	5	12	2	2	0
16820	1	1	2	1	63,826
16780	267	2,818	108	236	17,971
16640	62	413	2	6	311,201
16860	17	1	3	1	0
16850	18	1			1
Mean	89	435	21	64	56,622

[a]Last five digits of ADFG stream catalog number.
[b]Means of three to six samples; data from Brannon et al. (1995).
[c]Means of one to six samples.

Persistence of oil reservoirs along the sides of streams has been proven. Persistence in the stream deltas is not a unique occurrence for PWS. Studies of oiled mussel beds and beaches have demonstrated widespread persistence of oil in a variety of habitats other than stream deltas (Harris et al., 1996, Babcock et al., 1996, 1998, Thomas et al., 1999a; 1999b; Brodersen et al.,

1999). Contamination of mussels indicates that the underlying oil is biologically available. Further, these studies have also identified the oil as originating from the EVOS. The oil is weathered, but only to the extent that the low molecular weight mono- and di-aromatic hydrocarbons are absent; the oil often remains fluid and mobile if accessed through tidal flushing (overlying mussels), or by moving overlying rocks (Brodersen et al., 1999).

The persistent presence of oil in the deltas of oiled streams and the continued measurement of elevated embryo mortality suggested the possibility of continuing oil exposure. Government researchers speculated that oil in the streamside deposits was making its way into spawning redds via the movement of interstitial waters from the delta and through the streambed sediments. In this exposure model, the contamination would be more indirect (PAH coming from the sides) and would likely be intermittent, as the freshwater flow in salmon redds is interrupted on a rising tide for up to 4 hours (Helle, 1970).

In an attempt to validate the above model, government researchers (Rice et al., in prep.) monitored the movement of flourescent dyes from injection points in the deltas above the stream beds and near the high tide mark, at two oiled, intertidal streams in PWS. Flourescent dyes were injected into the sediment at approximately the high tide mark during low tide. On subsequent tides, water samples were taken from delta sediments, streambed sediments, and stream surface water. Dyes percolated through the sediments with both incoming and outgoing tides, and significant concentrations of dye were found in the streambed sub-surface sediments, the environment of potential salmon redds.

The two groups of researchers considered exposure of eggs and embryos from different sources. Brannon et al. (1995) expected direct contact exposure and measured contamination of streambed surface sediment. Murphy et al. (1999) measured persistent PAH concentrations in the sediments in the areas surrounding the streams, and thus the potential for exposure via contaminated interstitial water. Results from the flourescent dye studies indicate this is a viable alternative to direct exposure. The measurements by Murphy et al. indicate that the stream deltas

were recovering from oil contamination by 1995, but they were certainly not as clean as reference streams. Oil persistents in the stream deltas and can reach salmon redds via interstitial flow.

(2) Biological Evidence Of Oil Exposure To Pink Salmon Embryos: There are two types of biological evidence of oil exposure to embryos and alevins: (1) the elevated embryo mortalities in oiled streams discussed in the following section, and (2) the induction of P4501A enzymes in alevins sampled in oiled streams. Wiedmer et al. (1996) sampled alevins from spawning gravels of four oiled and five reference streams in spring of 1990 and 1991 (two brood years) and tested for P450IA induction. Induction was noted in 13 of 16 alevin samples collected from oiled streams but was not found in any of 7 alevin samples collected in the reference streams. Two years after the spill, oil contaminants were still biologically active and inducing a measurable P4501A response in alevins from oiled streams.

(3) Weathering, Toxicity, And Life Stage Sensitivity: Weathering is usually considered a good thing following a spill- less oil mass persists. The relevance to acute and long-term toxicity is not that simple. Weathering reduces acute toxicity of oil quickly as the single ringed aromatics evaporate readily from the oil mass (benzene, toluene, xylene for example). The loss of two ringed aromatics is faster than the 3-4 ringed compounds because biodegradation and dissolution occur at higher rates for the smaller hydrocarbons. Hence, weathering can affect the acute toxicity significantly in a short period of time. Prior to EVOS, researchers debated the relative contributions of different hydrocarbons to the toxicity in oils. The larger, mult-ringed compounds were known to be more toxic by orders of magnitudes (Rice et al. 1976; Black et al. 1983), but in short-term tests with WSFs, the larger compounds were not available to animals in the water column because of their relative insolubility, and hence their toxicity effectiveness was not considered as great as the one and two ringed aromatics. One and two ringed aromatics were presumed to be the compounds most responsible for the acute toxicity of crude oils.

Weathering does not affect long-term toxicity nearly as effectively as acute toxicity, because the larger 3-4 ringed aromatics are not taken away. There was little concern for long-term toxicity prior to EVOS, as there was little evidence of long-term toxicity from a spill. Persistence of the oil from EVOS, coupled with long-term effects to sea ducks, otters, and pink salmon renew the significance of long-term toxicity to an ecosystem. Enriched composition of weathered oil with 3-4 ringed aromatics was confirmed in the stream delta samples collected in 1995 (Figure 7 Murphy et al., 1999), and has been confirmed in the studies of long-term oil persistence in mussel beds. Bioavailability becomes a major issue with 3-4 ringed aromatics; they are 3-4 orders of magnitude more toxic than the mono-aromatics if they can be absorbed by animals.

Long-term toxicity is also heavily influenced by life stage sensitivity and biology, and pink salmon embryos demonstrate this interaction. In the 1970s, short term acute toxicity tests with WSFs determined salmon eggs to be quite tolerant (Moles et al., 1979), and the tolerance to short-term acute toxicity tests has been cited by Brannon and Maki (1996) as evidence that little harm could come to incubating embryos in the contaminated stream deltas following the spill. That would probably be true if the exposures were short-term, as in a few days. Embryos are usually thought of as sensitive life stages, but it can take time before developmental abnormalities are observable and before they affect the long-term survivability. The biology of pink salmon embryos is a slow developmental period (6-8 months), which permits a lengthy exposure and uptake period (access to the low solubility 3-4 ringed aromatics). Dependence on lipophilic yolk provides a mechanism for long term retention of the large aromatic hydrocarbons. The sensitivities of embryos to long term exposure (at part per billion levels) is covered in the following sections.

(4) Reconciliation Of Results: Relative to oil persistence and composition, the two groups of researchers collected complementary data, but not comparable data. The Murphy et al. (!999)

data on stream deltas indicates the persistence (through 1995) and potential for long-term exposures. Brannon et al. (1995) in-stream samples indicated that the exposures would be low, if at all, and they would probably be indirect. Neither group has measured in-stream water samples through a tidal cycle, when rising seawater would flood contaminated stream sides. Biological evidence of long-term exposure is indicated by the P4501A induction measurements in alevins from oiled streams, through 1991, when the last samples were collected by Weidmer et al (1996).

B. MORTALITY OF EGG AND EMBRYOS IN THE FIELD

Government and Exxon researchers differ greatly on the issue of direct egg and embryo mortality in oiled streams. Bue et al. (1994, 1996, 1998) found elevated mortalities in oiled streams through 1993 (Figure 7.), four years after the spill, and Exxon researchers, reporting no mortalities, have disputed these findings. The issue is significant because it would be a rare example of "new" damage in outlying years after a spill, as opposed to a continuation of the initial acute effect on a population. Such was the case in the long-term recovery of some sea birds that suffered population-level impacts in the first months of a spill, and required several generations to recover. Government- and Exxon-sponsored studies differ in findings, methods used, statistical power, and significance of the results.

(1) Direct Embryo Mortality – 1989 Through 1993: Bue et al. (1994,1996, 1998) measured elevated embryo mortality in oiled streams from 1989 through 1993. Mortality rates vary each year, but relative to non-oiled reference streams, the oiled streams had a significantly elevated mortality rate. Bue et al. hydraulically sampled embryos from spawning gravels at 14 locations along transects at three different intertidal zones and one zone above mean high water. Ten oiled and 15 reference streams were studied from 1989 to 1995. Hydraulic sampling is a standard

assessment for spawning success and embryo survival conducted by Alaska Department of Fish and Game for many streams in PWS, and these assessments were extended to the oiled areas in 1989. Mortality was still significantly greater in the oiled streams through 1993, but was not significantly different in 1994, 1995, and 1996. Mortality was again greater in oiled streams in 1997 (Craig et al., in press).

In contrast, Exxon researchers (Brannon et al., 1995) found no elevated embryo mortality in oiled streams, including some of the same streams sampled by Bue et al. The methods differed significantly between the two studies, even though some of the same streams were used. In the Brannon et al. study, fish from five oiled streams and four reference streams were spawned artificially, and eggs were incubated in small Whitlock-Viebert chambers (50 eggs per chamber) in the gravel of the same streams. The chambers were placed in four zones: three intertidal and one above the intertidal zone. No difference was noted in the proportion of live embryos recovered from the in-stream incubators in mid-November 1989. No further survival measurements were made by Brannon et al. (1995) after 1989.

(2) Comparison Between Field Studies Is Difficult: Reconciliation between the two studies will be difficult, because there are distinct differences in method and sampling design, statistical power, and viewpoint. Both studies used three intertidal zones below the high tide, plus the zone above that is not influenced by seawater at high tides. Bue et al. sampled redds in the spawning gravels by pumping random spots on a diagonal transect through each zone. Brannon et al. used freshly spawned embryos held in small chambers that were placed into the stream. Although some streams were used in both studies and the zones sampled were the same, the incubation of the embryos was different. There is no way to compare the two studies with respect to intra-gravel flow to the embryos or the distance of the embryos to the sides of the stream where known oil contamination persisted.

The statistical power was substantially lower in the Brannon et al. (1995) study. The

ADF&G study by Bue et al. (1998) examined more streams and more embryos than Brannon et al (1995) study (Table 2). The difference in statistical power is most evident when the number of eggs examined per stream (1,200 vs 12,160) or the total number of eggs from the oiled streams (6,000 vs 120,000) are compared. As noted by Petersen et al. (in press), initial design discussions in other Exxon studies has greatly limited statistical power and the ability of tests to detect differences when compared to government studies. The two research groups often looked at similar materials, but probably for different reasons. Bue et al looked for subtle differences overlain on a highly variable parameter, and employed enough statistical and design power to find damage if it occurred. Brannon et al. looked for lack of catastrophic effects, did not have as much statistical power, and were less likely to find damage if it were subtle.

Table 2. Comparison of streams and embryos examined by Bue et al. and Brannon et al.

Variable	Brannon et al.	Bue et al.
# ref streams	4	15
# oiled streams	5	10
# chambers or stations per zone	6	14
# eggs/embryos per zone	300	~3040
# eggs/embryos per stream	1200	~12160
area of stream sampled	*0.2 m^2	16.8 m^2
# of years	1 (1989)	9 (1989-1998)

* area restricted to position of 6 Whitlock-Viebert incubation chambers/zone

(3) Did Sampling Time Affect The Results?: Exxon researchers argue the measurements by Bue et al were flawed (Brannon and Maki, 1996). They contend that embryo sampling occurred consistently (2 weeks) closer to spawning time in the oiled streams as compared to the unoiled streams, and that this resulted in disturbing the embryos during a very sensitive period. Greater mortality in the oiled streams, they allege, was due to sensitivity of the embryos to physical shock during the field sampling. This hypothesis was examined by ADF&G and in the final report (Craig et al., in press), and the authors neither reject nor confirm it. Sampling in streams was based on run timing, but daily counts were not practical in all of the streams sampled. In

1991, more run timing data was gathered than in the other years. While the hypothesis of early sampling in oiled streams cannot be rejected, there was still a statistically significant effect related to oiling when sampling date was used as a co-variate. Oiled streams were still significantly affected by oil in 1991, and it is likely that the higher oiling years of 1989 and 1990 were also affected, even though run timing effects cannot be ruled out. Further, in 1993, fresh spawn was taken from returning adults to oiled and control streams, and the fertilized eggs were reared in a controlled hatchery environment. In this year, the oiled streams with elevated embryo mortalities had the same relationship with those reared in the hatchery, indicating that the effect was on the adults, and that run timing was not an issue with the oil effect. The weight of the evidence would indicate that elevated mortalities were caused by oil in the environment.

C. EFFECT OF OIL ON REPRODUCTIVE IMPAIRMENT IN RETURNING ADULTS

Continuing elevated embryo mortalities in oiled streams 4 years after the EVOS lead to the question of possible reproductive impairment in returning fish, where damage could be passed on to the next generation. Elevated embryo mortality in the freshwater zone above high tide in 1991, where the embryos were not likely to have been exposed to oil from the contaminated deltas, was the stimulus for this concern (Bue et al., 1996). There are three basic explanations for these findings: (1) the field measurements were flawed, and no damage was transmitted from the returning adults to the next generation, (2) returning adults had less reproductive viability because they were exposed as embryos in the stream (fall 1989), and (3) returning adults had genetic damage from exposure when they were embryos. Evidence of long-term damage from oil was rare, let alone damage that could be passed to succeeding generations. Because of the power of the sampling design and continuing embryo mortalities from year to year, the observations of elevated embryo mortality in the freshwater section were mostly believed by government researchers, even though there was a lack of overwhelming supporting data. The

returning adults in 1991 were from the first brood year (1989) that incubated a full cycle in the contaminated deltas of oiled streams. These observations stimulated further review and new studies.

(1) Controlled Hatchery Incubation Tests Of Reproductive Impairment: In two studies investigators spawned returning adults from oiled and non-oiled streams to test for genetic or reproductive viability problems. The spawn was incubated in a controlled hatchery environment where residual oiling in a stream was not a factor. Environmental variables, such as temperature and water flow, were also eliminated because the hatchery environment was identical for all eggs in the tests. Exxon and government researchers report differing results, even though some of the same oiled streams were used by both sets of researchers.

Brannon et al. (1995) conducted hatchery incubation tests in 1990 and 1991, and found no difference between fish from oiled and non-oiled streams. In 1990, eggs and sperm from 20 females and 40 males at each of two oiled and two reference streams were returned to a hatchery for long-term incubation (40 crosses per stream). In 1991, a like number of fish were used to supply fertilized eggs from five oiled and five reference streams. The returning adults in 1990 had no exposure to oil as eggs or embryos, but were presumably exposed as alevins nearing the end of development, and as fry emigrating out of PWS. The returning adults in 1991 could have been exposed as embryos for their entire natal history, when oil concentrations were at the greatest levels.

In contrast, Bue et al. (1998) measured elevated mortality in embryos when spawn was taken from adults returning to oiled streams in 1993 and 1994. They observed crosses from 30 female and 30 male pink salmon from each of eight oiled and eight reference streams in PWS in 1993 and 1994 (Figure 9). Embryos of each female were fertilized with sperm from each male (900 crosses per stream) and incubated in a hatchery. The pattern of elevated embryo mortalities in 1993 was the same as in the streams during hydraulic pumping surveys in October of that year,

the last year that elevated embryo mortality was statistically greater in the oiled streams. In 1994, elevated embryo mortality was not statistically significant in the streams measured by pumping in October, and likewise, elevated mortality was not measured in embryos when spawn was taken from adults returning to oiled streams and incubation was in the hatchery. The hatchery spawnings reported by Bue et al. (1998) were consistent with their field observations that found an oil effect in 1993 and no oil effect in 1994.

(2) Reconciliation Of Results:

Exxon researchers note that Bue et al (1998) hatchery studies had high mortality for a hatchery study while mortality in Brannon et al. study was low, indicating a problem with the Bue et al data. However, high mortalities occur in both the oil and reference crosses and are the result of greater amount of handling need to produce 900 crosses. This handling procedure is probably more consistent with the type of handling the embryos get naturally, was applied equally to all the crosses, and was a necessity of the experimental design.

Brannon et al. (1995) tests in 1990 and 1991 were low in statistical power, and it is also not surprising that Brannon et al. failed to find significant differences, because the effects were not catastrophic and required a high level of statistical power in the test design. Bue et al. again had significantly greater statistical power with more streams, more females, more crosses, and more eggs (Table 3).

Table 3. Comparison of controlled hatchery incubation studies

Viable	Brannon et al.	Bue et al.
# Reference streams	2 – 5	8
# Oiled streams	2 – 5	8
# females spawned per stream	20	30
# test crosses per stream	40	900
# eggs incubated per stream	~8000	~27000

Bue et al. used a mating procedure designed specifically to estimate mean viability, whereas the Brannon et al. design was aimed at determining the sources of variation in viability. The case for the Bue et al. study is further supported by the parallel results found in the field for the same

years, elevated mortality in 1993 but not in 1994. It is not surprising that Brannon et al did not find an effect in the 1990 brood year, for these returning adults did not have a lengthy exposure at all, and none during the embryonic stage.

Sample size becomes even more important in light of a pink salmon straying study involving both wild and hatchery stocks in PWS (Sharp et al., 1994). Hatchery contributions to 46 PWS streams surveyed daily ranged from 0% to 47%. In addition, they report a range of 9% to 53% straying in CWT wild stock recovered from three oiled streams and three reference streams in 1991. The smaller the sample size, the greater the impact would be of fish being captured in other than their natal stream. For at least some streams in PWS, there is up to a 47% chance that a female captured in a stream is of hatchery origin rather than wild stock, and up to a 53% chance that even if it is a female of wild stock, it is not in its natal stream (Sharp et al., 1994). Strayed fish would have an unknown exposure history compared to natal stream fish and would dilute the embryos of natal stream fish in the experiment.

D. GENETIC EFFECTS: ARE THEY REAL, ARE THEY SIGNIFICANT?

Genetic effects from oil exposure, if they occur, would be significant because the mutation could be passed on to subsequent generations long after the exposure event was over. Although abnormalities in the genome might result from oil exposure, two points should be emphasized: (1) severe negative genetic effects are going to cause mortality, and will be selected against and removed from the population relatively quickly, and (2) because negative genetic effects are going to cause increased mortality rates, the ability to detect genetic effects in a population is going to be restricted to the survivors, among whom the genetic damage is less severe and more difficult to measure.

The question of genetic effect arose due to two field observations: (1) elevated embryo mortality in 1991 in oiled streams above the upper oiled zone, and (2) the observation of elevated

embryo mortality in a hatchery, where the only possible oil exposure was in the adults harvested from oiled streams. Neither field observation can separate genetic effects from adult reproductive impairment, and hence, the initiation of limited studies to examine for genetic effects and reproductive impairment.

In controlled laboratory exposures of pink salmon eggs and embryos to crude oil, Seeb and Habicht (1999) failed to find macrolesion damage using flow cytometry technology. In contrast, Roy et al (1999) used polymerase chain reaction analyses of pink salmon embryos exposed to weathered North Slope crude oil and found somatic cell mutations in high frequency at mutational “hot spots” in genes such as *k-ras*. The authors note that these mutations have not been looked for in natural populations, and hence the heritability has not been confirmed. The laboratory test suggests the possibility, and coupled with the field observations of elevated embryo mortalitiy when direct oil exposures were not probable, it suggests that genetic damage may be a contributing factor to long-term effects.

The evidence of reproductive impairment is supported by the field observation of elevated embryo mortality in the freshwater zone in 1991 (Bue et al., 1996) and by the hatchery tests in 1993 and 1994 (Bue et al., 1998). Both tests (field and hatchery) revealed significant, but not catastrophic, mortality among the offspring of fish that presumably incubated in oiled gravel. However, neither test could establish whether eggs or embryos were non-viable as a result of the parent's inability to form viable gametes or from inherited genetic effects. The distinction is important because the former explanation will influence only one generation, whereas the latter may persist in the population for multiple generations. The preponderance of evidence, at this time, suggests that mortality resulted from the parent's inability to form viable gametes. The measurement of oil along the sides of streams plus laboratory evidence of sensitivity embryos suggests that continuing exposures are sufficient to cause "new" impacts to succeeding generations, and damage in outlying years does not depend on a genetic model that is passed to succeeding generations. Reproductive impairment was also suggested by Marty et al. (1997),

who reported abnormal histopathology of gonads in pink salmon alevins after lengthy low-level exposure (ppb PAH) in laboratory tests. However, although oil is a known clastogen (Al-Sabti, 1985) and embryos exposed to oil during incubation have shown DNA sequence alterations (Roy et al., 1999), exposure trials measuring genetic impacts have not been particularly successful in demonstrating an effect (Brannon et al., 1995). Genetic impacts versus reproductive impacts are not mutually exclusive, and both mechanisms were likely to have been operating in the first years after the spill.

E. CONCLUSION

Field studies of embryos incubating in freshwater streams of PWS demonstrated significant and persistent evidence of crude oil effects at the individual stream level. The combination of long-term presence of biologically active oil in stream deltas through 1995 coupled with observations of elevated embryo mortality through 1993 are clear evidence of biological effects of oil contamination. Oil collected from the stream deltas was significantly weathered, which led to increases in the long-term toxicity of the persistent oil fractions. The issue of run timing can not be rejected as a contributing factor in the estimates of mortality in several of the years, but the effect of oil as a causal factor was confirmed in 1991. This was the year with the most stream-specific run timing data. The two earlier years were more contaminated, and an oil effect was even more likely. The pattern of elevated embryo mortality in 1993 was also observed in controlled hatchery experiments measuring mortality in embryos of eggs taken from adult salmon returning to oiled streams that year. This was probably the result of parental inability to produce viable gametes rather than genetic damage. The statistical power of government tests was actually greater by an order of magnitude, hence the weight of the evidence is for an oil effect on incubating pink salmon embryos in oiled streams for several years after the spill.

IV. LAB STUDIES ON SENSITIVITY OF EGGS/EMBRYOS

Bue et al. (1996) initially found elevated embryo mortality in the field without much evidence of exposure and without a biological mechanism. In fact, the acute toxicity studies of the 1970s had demonstrated tolerance by embryos to short-term exposures to WSF and were used by Exxon researchers to criticize the results of the field studies by Bue et al. Long-term laboratory exposures were initiated by government researchers to determine long-term sensitivity of embryos to a weathered oil fraction. These studies found sensitivity at part per billion concentrations and higher than expected toxicity of weathered oil. Further tests with embryo exposures found delayed effects on marine growth and survival of returning adults. Exxon researchers never attempted to dispute these studies by conducting their own laboratory studies.

A. LONG-TERM SENSITIVITY OF EMBRYOS TO WEATHERED CRUDE OIL

(1) Pink Salmon Embryos Laboratory Tests: Pink salmon embryos were sensitive to long-term exposure to weathered oil (Marty et al., 1997). After long-term exposure in gravel contaminated with weathered Prudhoe Bay crude oil, sublethal biological effects were measured at water concentrations as low as 4.4 ppb PAH. Larvae were sampled at 4 weeks before emergence, at emergence, and 13 days after emergence. Hydrocarbon analysis revealed that tissue uptake of PAH was slow, but significant, and highest concentrations were found when there was a significant amount of yolk present. The outer embryo membranes were definitely porous enough for high molecular weight compounds to pass through, and the high lipid content of the yolk is an excellent tissue for high affinity and retention of large multi-ringed PAH. Oil-related effects noted included induction of cytochrome P4501A, development of ascites, retarded development, and increased mortality. As opposed to control fish, oil-exposed fish of the same age and emerging at the same time had more yolk and hepatocellular glycogen, increased

apoptosis of gonadal cells and midventral skin cells, and less food in the gastrointestinal tract. At the time of these tests, the biological significance of these effects was not known, but later tests have indicated delayed impacts on growth, marine survival, and the gonads. Gonadal apoptosis may be associated with the reproductive impairment measured from field-sampled fish by Bue et al. (1996).

The long-term sensitivity of embryos was confirmed again in tests using weathered oil by Heintz et al. (1999). Sensitivities in the part per billion range were re-confirmed, and the most toxic test was with a more severely weathered oil (Figure 10). Using the most weathered oil in their study, a peak high concentration of aqueous PAH of only 1.0 ppb resulted in significantly greater mortality than in controls. Biological effects were the same when embryos were incubated in this lab study on oiled gravel, or in water effluent from oiled gravel. They concluded that PAH accumulation must, therefore, be mediated by aqueous transport and that direct contact with oiled rock is not necessary. This controlled-exposure study supports the hypotheses that oil in the streamside deltas could supply low concentrations of oil to embryos in salmon redds, and that embryos do not have to be in contact with oiled sediments.

These two laboratory studies (Marty et al., 1997; Heintz et al., 1999) are significant for two reasons: (1) both studies measured sensitivities of pink salmon embryos to lengthy exposures, and (2) weathered oil is more toxic than previously assumed. These studies support the possibility that part per billion PAH exposures from adjacent oil deposits in stream deltas could cause the elevated embryo mortality measured in the field through 1993.

The part per billion sensitivity reported appears to have more to do with the life stage (developing embryos) than with the species. In a study similar to the Heintz et al. study, but with a different species, Carls et al. (1999) exposed Pacific herring embryos to the effluent from weathered oil contaminated gravel. Sixteen day exposures to initial aqueous concentrations of 0.7 ppb PAH caused malformations, genetic damage, mortality, decreased size, and inhibited swimming. Total aqueous PAH concentrations as low as 0.4 ppb caused sublethal responses

consistent with premature hatching. When exposure was to less weathered oil, the lowest observed effective concentration was 9.1 ppb, as compared to the 0.4 ppb for more weathered oil. This value, 0.4 ppb, is similar to the lowest concentration of weathered oil, 1.0 ppb, noted to affect pink salmon (Heintz et al., 1999).

(2) Delayed Effects Of Embryonic Exposure To Crude Oil: The toxic effects of oil on developing embryos may not be expressed until long after the exposures have ended. Heintz et al. (1996) compared marine growth rates of pink salmon exposed to and not exposed to weathered oil during the embryo stage, and reported that growth rates of fish incubated in an aqueous PAH concentration of 1.0 ppb were decreased by more than 10%. Growth rates in that study were measured 4 - 6 months after the exposures ended and were determined from observations of more than 150 fish per dose. In a similar study, Heintz et al. (in press) incubated embryos in clean water or water effluent from gravel contaminated with weathered oil with an aqueous PAH concentration of 5.4 ppb. More than 70,000 survivors from each exposure were tagged and released, and small numbers were retained to evaluate early marine growth rates. The exposed fish had lower average growth rates during their first 10 months after emergence. Although returning adults were the same size, average survival of the exposed population was 15% lower than the unexposed population. In Figure 8, doses of oil that cause delayed effects on growth in the first 4-6 months in seawater are compared to the doses exposed to embryos that resulted in delayed impacts on survival. Doses of 18 ppb caused significantly less growth, and doses of 5.2 ppb caused significantly reduced survival compared to unexposed fish. These data suggest the increased mortality in the exposed population probably resulted from its greater proportion of slower growing individuals.

These delayed effects change our perception of the overall toxicity of oil to developing embryos. PAH concentrations in the studies described above decreased to about 5% of their initial levels within the first 40 d of exposure. Apparently, damage was acquired during early

development. The resulting delayed effects indicate that embryos have limited ability to repair the acquired damage, which apparently has profound effects later in life. In fish, sublethal response that may not be noted during exposure may ultimately translate into mortality. Thus, we shall underestimate the toxic effects of oil on developing embryos unless we also account for lethal or sublethal effects that are not expressed until later in life, and recognize the greater impact of chronic effects.

B. TOXICITY OF AND SENSITIVITY TO WEATHERED OIL

Recent studies beg the question of toxicity of weathered oil or high sensitivity of the embryo life stage. These studies demonstrate that the toxicity paradigm for crude oil has changed since the 1970s, from short-term LC50 determinations and acute effects to long-term effects, from part per million to parts per billion toxicity. This change is due to two factors- weathered oil is more toxic and embryos are more sensitive than previously thought.

(1) Weathered Oil Is Toxic: Weathering processes have been known and understood for some time and are critical to the changing oil mass in the early stages of a spill (Wolfe et al., 1994; Wolfe, 1995). The lighter compounds, including mono-aromatics, which make up the bulk of the aromatic hydrocarbons in crude oil, are lost rapidly to evaporation. These compounds are highly mobile, in both air and water, and from a short-term acute perspective, they are the most feared compounds. They can be inhaled by the surface birds and mammals, and their effects can be lethal and rapid. In contrast, the multi-ringed aromatic hydrocarbons are much larger, more difficult to dissolve, slow to degrade, and make up only a small percentage of the aromatics. However, they can persist a long time in the environment, and they are orders of magnitude more toxic than 1-2-ringed aromatic hydrocarbons. In the 1970s, most concern for chemical toxicity to subsurface animals was to the 1- and 2-ringed aromatic hydrocarbons- they were toxic, abundant,

and more likely to be bioavailable. The larger 3- and 4-ringed compounds were known to be orders of magnitude more toxic (Rice et al, 1977, Black et al., 1983), but their availability was always downplayed.

Weathered oil was usually considered less toxic and less bioavailable; thus the findings of increased toxicity may appear confusing. When oil weathers, the lighter compounds are lost (evaporation, degradation), and the total mass decreases in volume and toxicity potential. However, if the least-toxic compounds are lost, then the resulting mixture is more toxic on a per unit mass basis because there is a higher proportion of the high-toxicity compounds in the mixture. At some point, if the compounds are tied up in a solid matrix, like coal or asphalt, they are not biologically available under normal circumstances, and their toxicity potential is reduced considerably.

As crude oil weathers, the composition shifts to a higher percentage of high molecular weight PAH. The toxicity of the remaining oil increases when compared on a unit of mass basis. Short and Heintz (1997) modeled weathered *Exxon Valdez* crude oil from a series of samples and quantified the shift in aromatic hydrocarbon composition. Heintz et al. (1999) demonstrated this shift in water exposures and in salmon embryo uptake, and presented evidence of greater toxicity when equivalent doses had higher proportions of the 3-4 ring compounds. In the effluent from oiled gravel, naphthalenes constituted 60% of the total PAH and phenanthrenes and chrysenes only 21% when the oil had a weathering factor of 0. With weathered oil (weathering factor = 4.9), naphthalenes accounted for only 5%, whereas phenanthrenes and chrysenes made up 64% of the total PAH.

The effect of a composition shift to the toxic 3-4 ringed aromatics on the relative toxicity of a solution is dramatic. The lowest effective concentration of the most weathered oil for mortality of pink salmon embryos was 1.0 ppb (Heintz et al., 1999), whereas effective doses are more than an order of magnitude greater with less weathered oil. The importance of composition in determining relative toxicity is even more dramatic when comparing toxicity of WSF and

weathered oil. Toxicity for pink salmon fry and for Pacific herring embryos is in the 1200-1500 ppb range of aromatics for WSF solutions (Carls. 1987, Moles, 1998) compared to about 0.4 -5 ppb aromatics in weathered oil (Heintz et al., 1999; Carls et al., 1999). WSFs have aromatic compositions dominated by 1-2 ring aromatics (82% mono- and 18% di-aromatic hydrocarbons; Carls et al., 1999) in contrast to weathered oil effluents, where phenanthrenes and chrysenes made up to 34 - 51% of the total PAH at the 0.4 ppb dose.

The possible contributory effects of biodegradation of PAH to toxicity noted by Middaugh et al. (1996, 1998) and Shelton et al. (1999) or photoactivation noted by Little et al. (2000) were not measured in the above studies. Possible enhanced toxicity of PAH due to these processes should not be ignored, however, both biodegradation and photoactivation-enhanced toxicity require the presence of the high molecular weight PAH.

(2) Sensitivity Of Embryos To PAH: Embryos are sensitive to aromatic hydrocarbon exposure, but because of their biology, their sensitivity can be hidden for some time. Early tests in the 1970s to acute WSF exposures found embryos relatively tolerant, but these tests did not hold the embryos for sufficient time after the exposures for effects to become visible. The experiments reported by Marty et al. (1997) and Heintz et al. (1999) demonstrate sensitivities at part per billion dose levels of PAH. These embryos were exposed for long periods, permitting more time for uptake and for impacts to become measurable. Outer embryo membranes are permeable, and hydrocarbon uptake occurs, although slowly (Heintz et al., 1999). Embryo yolk is lipid rich and has a high capacity to absorb and retain aromatic hydrocarbons, even when exposure concentrations are low.

Embryos may be sensitive at the early stages of development, but there are low concentrations of PAH in the yolk during this time. The most likely time for maximum vulnerability is after a period of time, when yolk concentrations of PAH are higher, yolk is being consumed, and organs and tissues are starting the more complex process of differentiation.

Damage to DNA at this stage will be passed on to all of the daughter cells stemming from the damaged cell. The deformities observed by Marty et al. (1997) appeared to be random and additive; a specific developmental stage or process was not damaged. Some damage is not critical to the developmental process and will have little impact until much later in life, e.g. gonad damage. The delayed impacts on growth (Heintz et al., in press) are examples of subtle non-visible damage having a significant delayed effect (decreased growth can result in increased predation and poorer survival).

Any test with embryos needs to have a long holding period for damage to become detectable. Much of the damage is hidden until the various organ systems develop a level of interdependency; in early embryo stages, damage is not readily visible, and the embryo appears to be developing normally. Holding for delayed impacts was not done with the early tests in the 1970s, and has been shown to be an important requirement of an experimental design in understanding oil effects.

C. CONCLUSION

Embryos and larvae of salmon are far more sensitive to oil than previously believed, especially after long-term exposure to heavily weathered oil. The long-term sensitivity of pink salmon embryos incubating in oiled gravel was confirmed in two rigorous laboratory experiments. Larvae had histopathologic abnormalities, increased induction of detoxifying enzymes, and increased mortality. Using heavily weathered oil, increased mortality of pink salmon larvae was noted at concentrations as low as 1 ppb. Similar experiments with herring embryos in saltwater noted increased mortality at slightly lower concentrations of weathered oil, suggesting that the results are not unique to pink salmon or fresh water. These toxic effects were often not evident until after the exposures ended, sometimes becoming evident during initial fry growth or in marine survival.

V. SIGNIFICANCE RELATIVE TO PRINCE WILLIAM SOUND

The preponderance of evidence indicates significant damage to pink salmon numbers in the early years, caused by reduced survival of embryos in oiled stream deltas, and by reduced growth (and thus survival) of fry in contaminated marine waters in 1989. These losses were significant to oiled streams, as estimated by the Geiger et al. (1996) model, but they were not catastrophic to the entire fishing district (including all of PWS, hatchery and wild fish, oiled and non-oiled streams). In the beginning of the spill, there was concern at the district level, as even the fish spawned in non-oiled parts of the sound would probably pass through the oiled areas in the southwestern district of PWS. The population crash in 1992 and 1993 fueled the speculation that oil had significant district-wide impacts.

A. POPULATION CRASH OF 1992 AND 1993- WAS IT OIL?

The population crash in 1992 and 1993 was significant to PWS (see Figure 1), but the linkage to oil is circumstantial at best. Evidence for the linkage to the oil spill is indirect: (1) a crash occurred in PWS, but not in any other district in Alaska, (2) oil effects at the individual stream level continued to be measured, and (3) oil was still evident in some beaches and stream deltas. If there was a direct toxicological effect of oil to pink salmon embryos because of oil persistence and embryo sensitivity, then the oil effect would be principally to the wild population from PWS, and not to the hatchery fish. Hatchery fish could have been exposed to oil when fry migrated from PWS in 1989, but all evidence indicates that marine exposures were non-existent after 1989. Hatchery fish also had poor returns in the same years, indicating that marine survival in the early seawater juvenile stages was responsible for the decline in both hatchery and wild stocks in 1992 and 1993. An indirect effect of the oil spill on “ecosystem” support for pink salmon is possible, but purely speculative at this point. The coincidence that a similar crash in

the herring of PWS occurred a year later in the same location is suggestive, but because the population crashes occurred 3-4 years after the spill, it is difficult to prove any connection.

B. LONG-TERM EFFECTS OF THE SPILL ON INDIVIDUAL STREAMS

Elevated embryo mortalities measured by Bue et al. through 1993, coupled with the availability of oil in stream deltas (Murphy et al., 1999), and the sensitivity of embryo stages provides convincing evidence that impacts at the individual stream level were significant and persistent. The impacts may have been much more significant, and much more evident, if oil impacts were not mitigated by two important factors. First, marine survivals of pink salmon juveniles were approaching or exceeding record levels in those years; food supply was abundant, and growth was good. Second, if specific stream populations were significantly affected, hatchery and wild strays would probably re-populate a stream quickly. High straying rates have been observed for pink salmon in PWS (Sharr et al., 1995), and because hatchery fish were insulated from early embryo contamination, their dominant production during those years would confound any interpretation of damages to wild populations based on numbers of returning adults.

Exxon researchers never conceded that specific stream impacts occurred, but argue that if they did occur, they were insignificant at the population level. However, given the straying level from both hatcheries and non-impacted wild streams, suppressed survivals in individual oiled streams could easily be obscured by non-impacted fish. Salmon are known for their homing ability, but straying may be more common for pink salmon relative to other salmonids that have multiple year classes. In pink salmon, where returning adults are restricted to one year class, some level of straying is thought to be a protective strategy (Quinn, 1984). This may be the case in PWS, where much higher straying rates have been observed relative to other geographic regions (Thedinga et al., 2000). This may be due to the environment in PWS that is geologically active (e.g. 1964 earthquake), and dominated by numerous short intertidal stream

systems; Thedinga et al. (2000) observed twice the straying from an intertidal-spawning population of pink salmon compared to an upstream-spawning population. Only a third of the streams in the southwestern district were oiled, leaving twice as many non-oiled streams to supply some stray fish, along with a large number of hatchery fish. All hatchery fish produced in PWS pass through the southwestern district and can contribute to the straying of fish to the oiled streams.

Given the short life cycle of pink salmon (2 years) and the annual infusion of strays from the large hatchery production, it is not surprising that the pink salmon populations in the streams of PWS rebounded quickly. One has to wonder what the impacts would have been with intertidal-dominated populations, without hatchery supplementation occurring independent of the spill. One also has to wonder what the impacts would have been during the 1970s when population numbers were at historic lows. Damages to populations at the stream level and at the region level may have been more easily measured under either of those scenarios.

VI. CHANGING PERSPECTIVES ON OIL TOXICITY

A. OIL TOXICITY PARADIGM SHIFT BETWEEN THE 1970s AND 1990s

The change in composition of the oil during weathering, and a resultant change in toxicity, is one of the major causes of conflicts in the interpretation of data from various studies. The toxicity data available at the time of the oil spill, and cited in studies detailing the effects of the EVOS, were collected using water-soluble or water accommodated fractions of crude oil. The tested fractions were prepared by gently mixing crude oil with water to provide a fraction that contained the most-soluble substances for use in short-term (96 h) toxicity studies (Rice et al., 1977, 1979, 1984). These un-weathered oil/water solutions contained high percentages of low molecular weight hydrocarbons (naphthalenes and below) and low percentages of the relatively insoluble, high molecular weight PAH (Table 4). However, it is the higher molecular weight PAH, which are relatively abundant in weathered oil, which are the most toxic; toxicity increases as the number of rings increases (Korn and Rice, 1981; Black et al., 1983).

Table 4. Oil toxicity paradigm shift.

	1970s	1990s
Length of exposures	4 day	7 months
Type of exposure	WSF	weathered crude oil
Dominant aromatic HC	1-2 rings	3-4 rings
Effects measured	survival	survival, deformities, growth, adult returns
Effective concentrations	1 ppm	1 ppb
Relative solubilities	high	orders of magnitude lower

Part of the paradigm shift is the issue of persistence. Persistence of weathered crude oil in certain environments exceeds expectations and has been demonstrated in the field- oiled mussel beds, some beaches, and some deltas of salmon streams. Persistence coupled with long-term

exposures and the sensitive biology changes the toxicity paradigm.

Brannon et al (1995) cited the acute studies of the 1970s (Rice et al., 1975, 1979, 1984; Moles et al., 1979, 1987)) as evidence that embryos were tolerant, and that low level exposures in the field could not account for the elevated embryo mortality measured by Bue et al. (1996, 1998) in the years after the spill. The acute LC50s measured with WSF were high, exceeding 1000 ppb of hydrocarbon in the water. In these short-term tests, embryos were more tolerant than alevins and fry. The long-term tests of the 1990s demonstrated that the tests of the 1970s with WSF were not appropriate for embryos. The exposure solution was not appropriate for the kinds of exposure that occurred; but most importantly, the tests were too short, and with no long-term holding period for embryos, negative impacts were not measured. Brannon et al. used these inappropriate data to support their arguments.

B. SIGNIFICANCE OF THE OIL SPILL TO URBANIZED ESTUARIES

The EVOS did not occur in an urbanized estuary, but the long-term impact findings are relevant to these habitats. The combination in some intertidal zones of low level contamination with weathered crude oil and with sensitive embryo stages causes concern for estuary habitats in urbanized areas, where long-term chronic contamination with PAH is an everyday occurrence. Pink salmon do not occur in San Francisco Bay or Boston Harbor, but other species do. Species that pass through some of these polluted estuaries have some level of exposure and risk, but not so much as those species that use these habitats for reproduction. Sensitive life stages, such as fish embryos, are likely to be impacted in these types of environments.

For 50 million people, the equivalent of one *Exxon Valdez* oil spill occurs each year. Two studies have estimated the amount of highway runoff on a per capita basis to be approximately one quart of oil per year (Eganhouse et al., 1981; Hoffman et al., 1983). One quart of oil per person times 50 million people is equal to the estimated spill of the *Exxon Valdez*. In an urban estuary such as San Francisco Bay, chronic runoff is a daily event: significant

concentrations of PAH can be found in water, sediment, and bivalves (SFEI, 1999). Water concentrations as high as 0.5 ppb PAH have been measured at times; about half the concentration of the lowest effective dose in the pink salmon toxicology studies. For long-term exposures to embryos and larvae, there would appear to be little safety margin, if any, between the highest levels of PAH found in San Francisco bay and the effective concentrations that are toxic over long periods of time. In urban estuaries such as San Francisco Bay, the problem is exacerbated by pesticide runoff especially at times of storm runoff (SFEI, 1999) Many other urban estuaries are also under chronic pollutant stress, and as the San Francisco Bay report demonstrates, urban estuaries are also assaulted by a host of other types of pollutants, such as heavy metals and pesticides. These chronic low level exposures are likely to have impacts, although some organisms may have adapted over time and cope with chronic PAH exposures. In PWS, there was less background PAH and the EVOS was an acute event for which pink salmon and other species were not genetically prepared.

Non-point source chronic pollution differs from a major spill: major spills like the EVOS are a one-time, acute event, with some level of diminishing persistence, compared to chronic input of many low level events occurring over a year in multiple watersheds. However, 50 million people will spill the equivalent of an EVOS this year, next year, and the year after. For this reason, urbanized areas have an insidious pollution potential. No single event is notable. There is not a mass die off, with floating carcasses. Rather, there may be continued erosion of populations, and over time, when coupled with other events such as hard winters, filled in wetlands, over-fishing, increase in predators, decrease in food availability at a critical life stage, or recruitment percentage decrease, may lead to extinction or gross reduction of the species from those high-impact environments. The species with a life history strategy that uses estuaries for reproduction will be hardest hit.

We hope this data synthesis will enable the reader to form an educated opinion of the effect of the EVOS on the pink salmon of PWS. In forming those opinions, it will be helpful to

consider the power built into the design of the studies, the toxicity of the PAH to which the life stages of pink salmon were exposed, and the laboratory studies conducted to verify field data. Although arguments put forth by government and Exxon researchers to explain their respective results from field studies may be inconclusive and unresolved, the reader should find no such ambiguity with results of laboratory studies with weathered oil. Comparison of PAH toxicity based on exposure to WSF (mono- and di-aromatic hydrocarbons) at part-per-million levels should not be made with the toxicity of weathered oil at oil spill sites (high molecular weight PAH) effective at part-per-billion level during intermittent, long-term exposures. Toxicity levels at approximately 1 ppb for the early life stages of both pink salmon and Pacific herring (Heintz et al., 1999; Carls et al., 1999) should be used as a basis for the re-examination of PAH water quality standards. Toxicity effects at the ppb level should guide assessment and studies of future oil spills.

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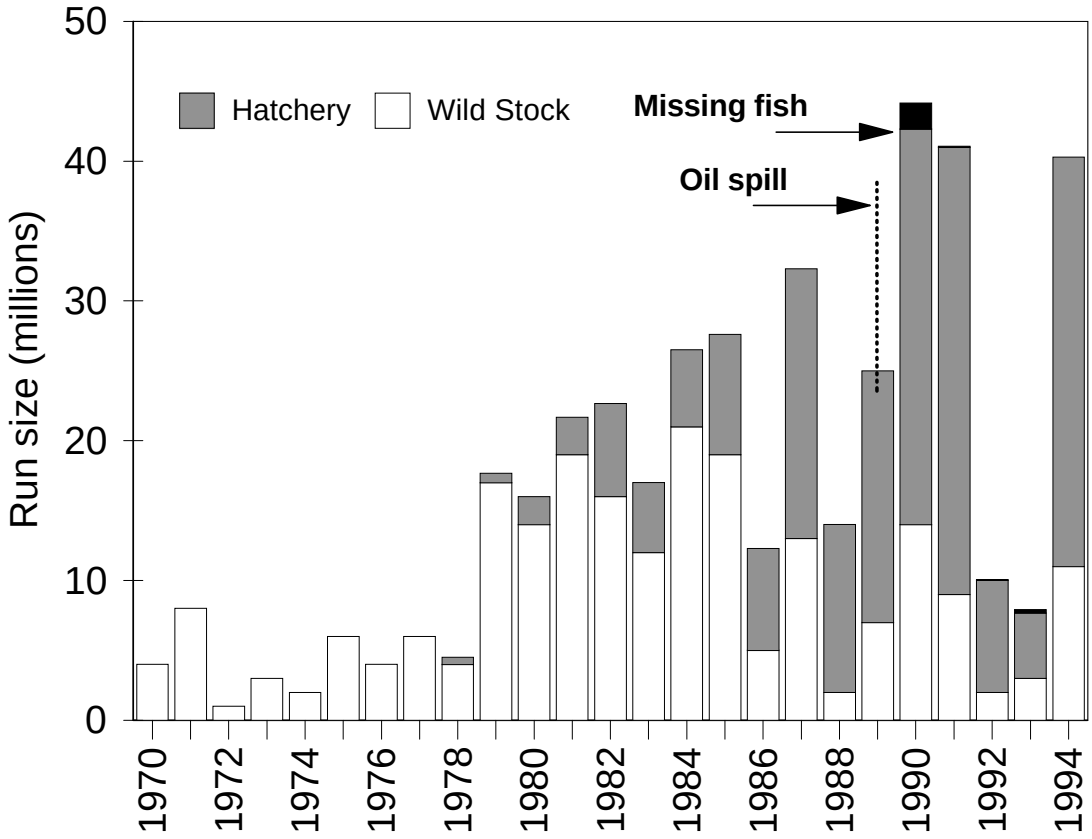

Figure 1. Wild and hatchery production of pink salmon in Prince William Sound before and after the spill

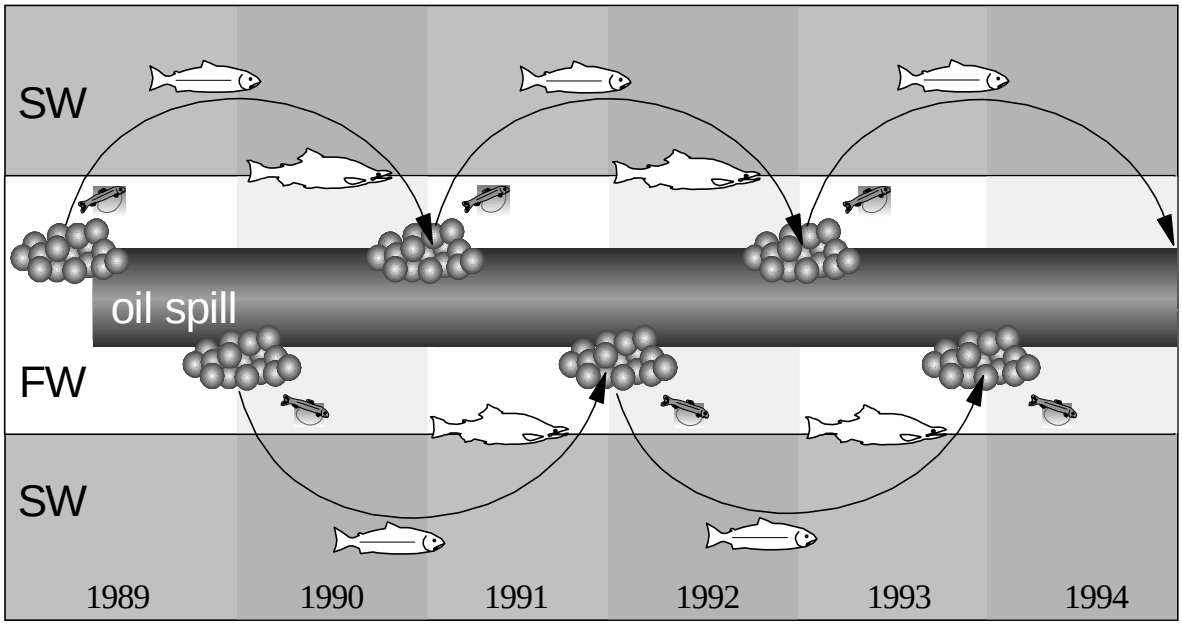

Figure 2. Two year life cycle of pink salmon, with odd and even year stocks, is pictured relative to the 1989 oil spill, indicating life stages that could have been exposed to oil in intertidal and marine habitats.

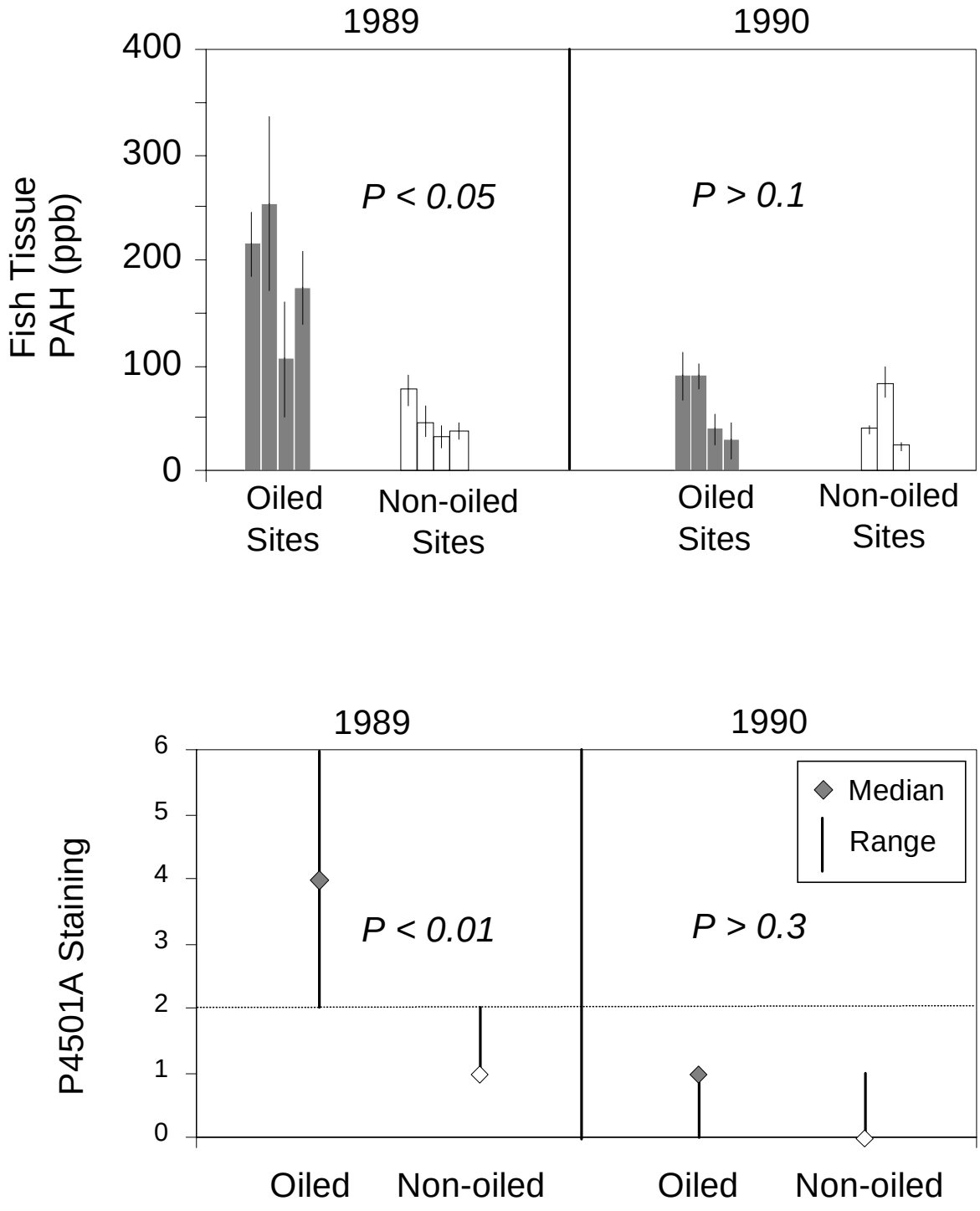

Figure 3. Evidence of oil exposure in 1989 in pink salmon fry collected from Prince William Sound: tissue concentrations of PAH and measurement of P4501A induction (data from Carls et al., 1996a).

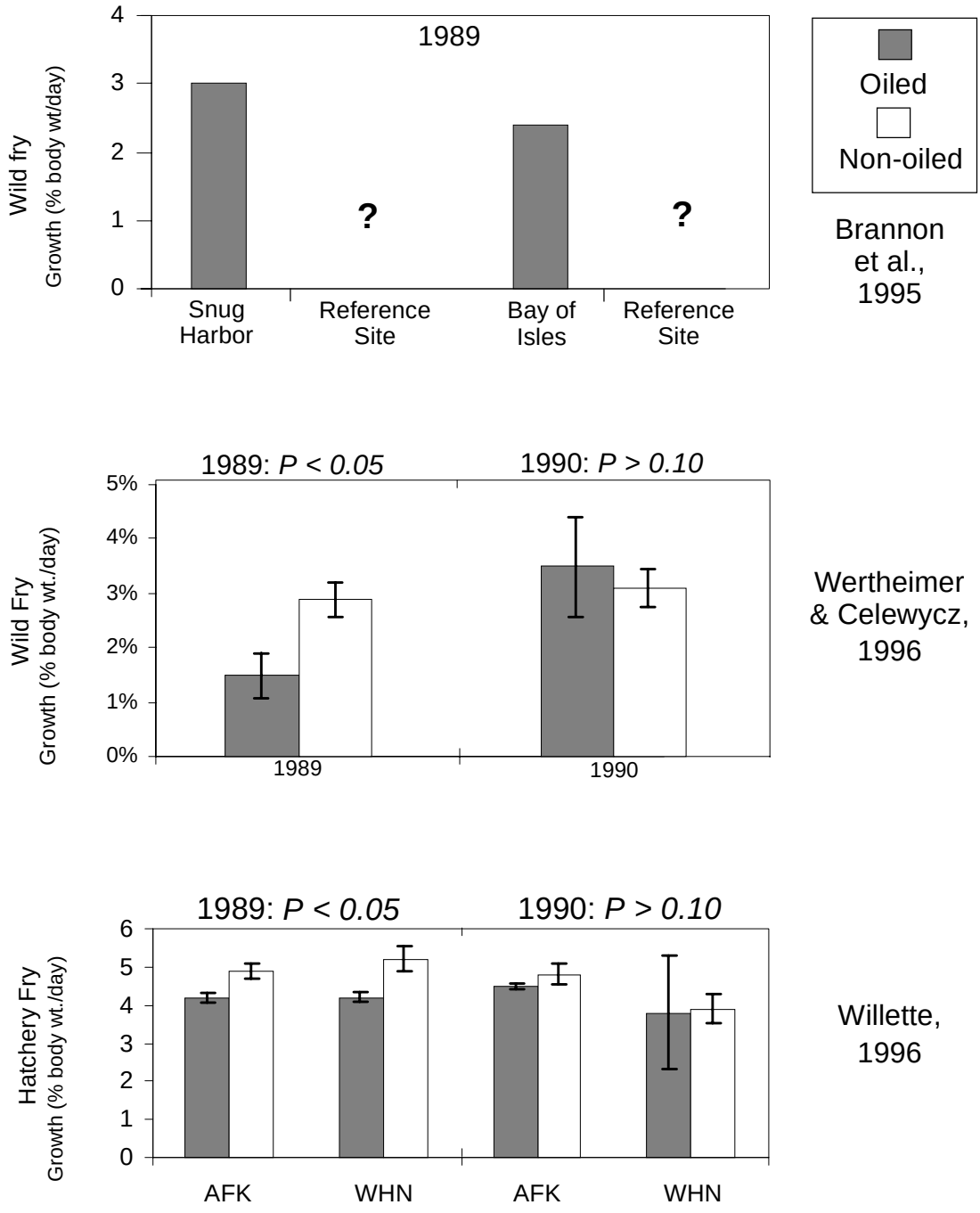

Figure 4. Comparison of fry growth rates between oiled and un-oiled areas by Exxon and Trustee researchers in summer 1989 (data extracted from Brannon et al., 1995, Wertheimer and Celewycz, 1996, Willete, 1996). AFK identifies fry from Armin F. Koernig Hatchery, WHN identifies fry from Wally H. Noerenberg Hatchery.

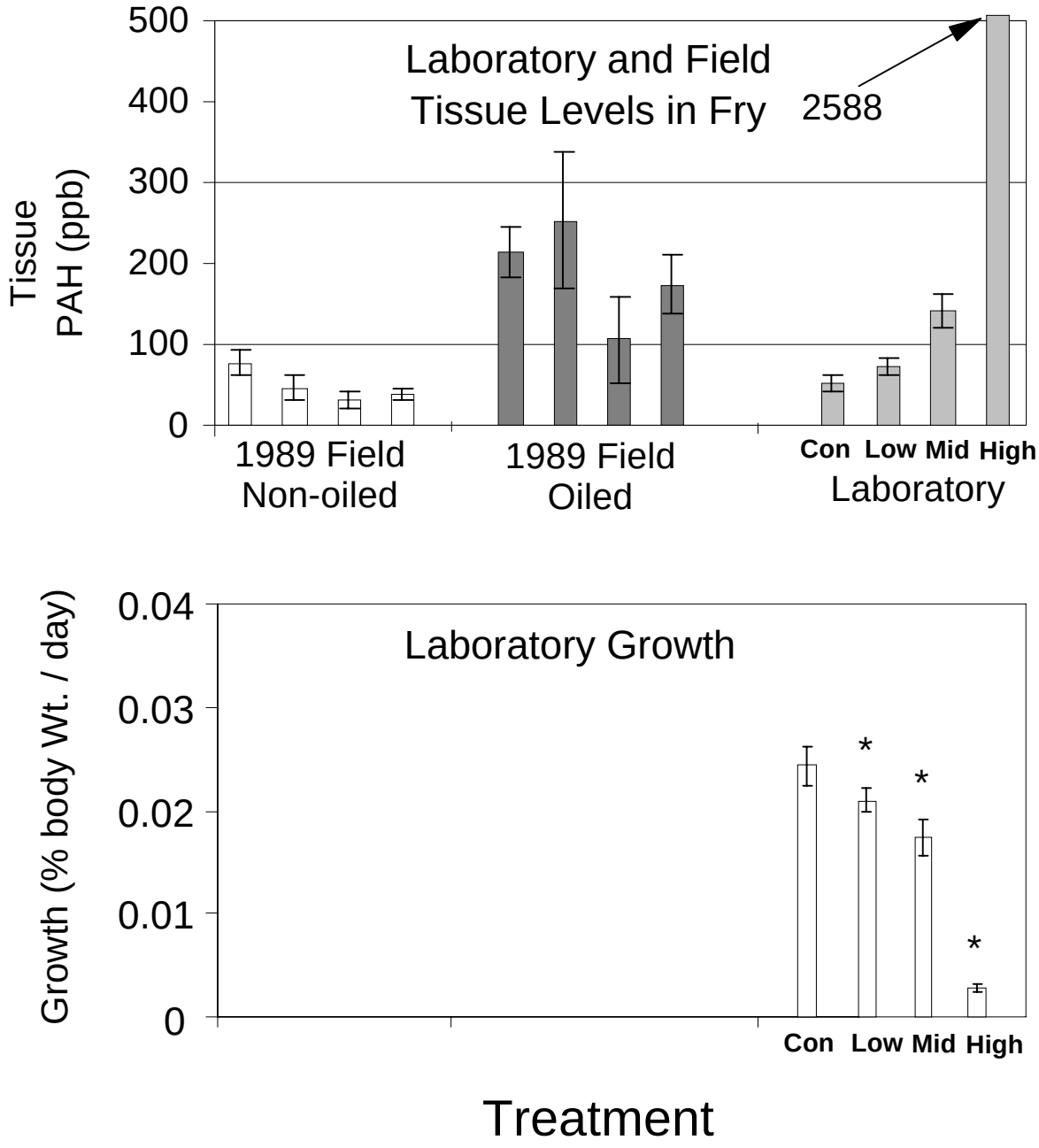

Figure 5. Comparison of aromatic hydrocarbon tissue loads and growth in fry exposed to oil in the laboratory, and tissue PAH loads in fry collected in PWS in 1989 (data from Carls et al., 1996a and b).

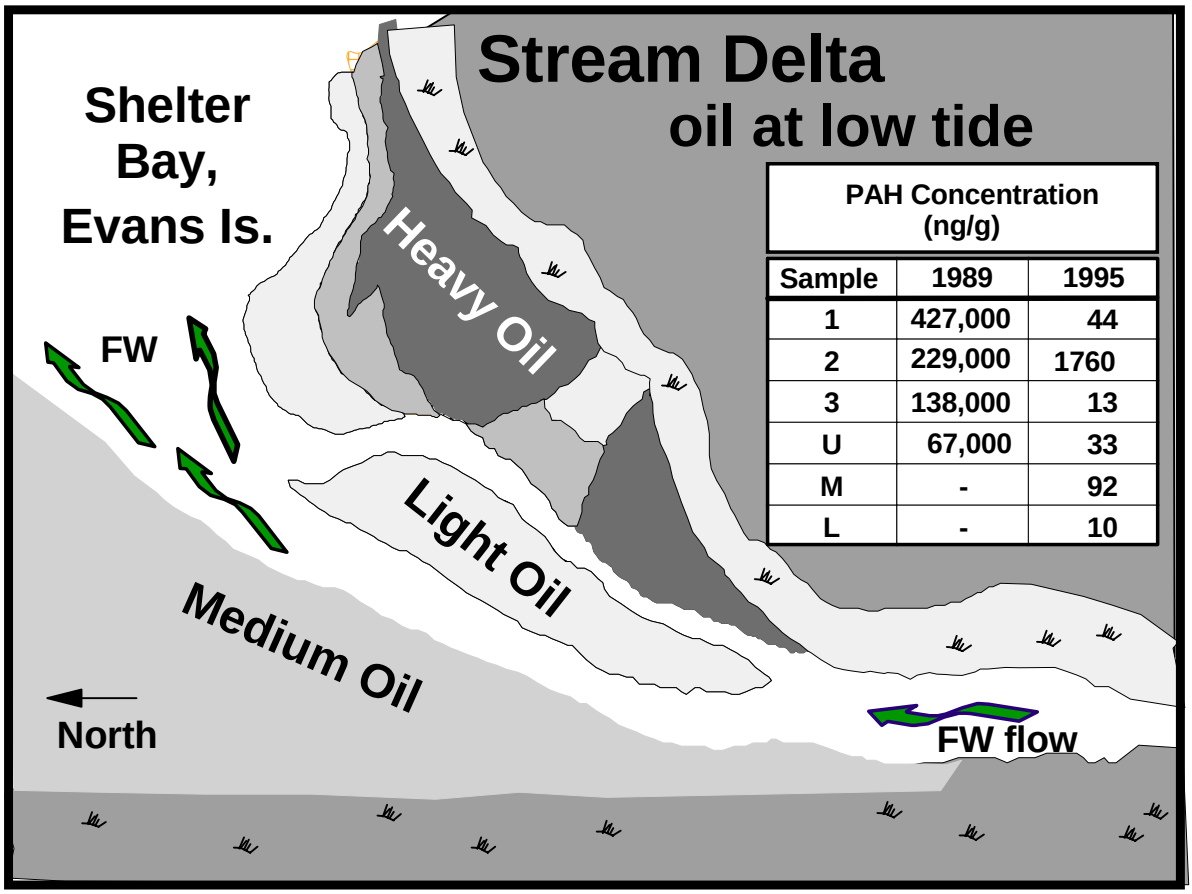

Figure 6. Map of the stream delta at Shelter Bay (stream no. 16613) in Prince William Sound, Alaska, showing distribution of observed oil in November 1989 (after the March 1989 *Exxon Valdez* oil spill) and location of fixed sampling plots (from Murphy et al., 1999, reprinted by permission). Table insert shows total polynuclear aromatic hydrocarbon concentration (PAH) in sediment from each sampling plot in 1989 and 1995. Original map was drawn by the Alaska Department of Fish and Game.

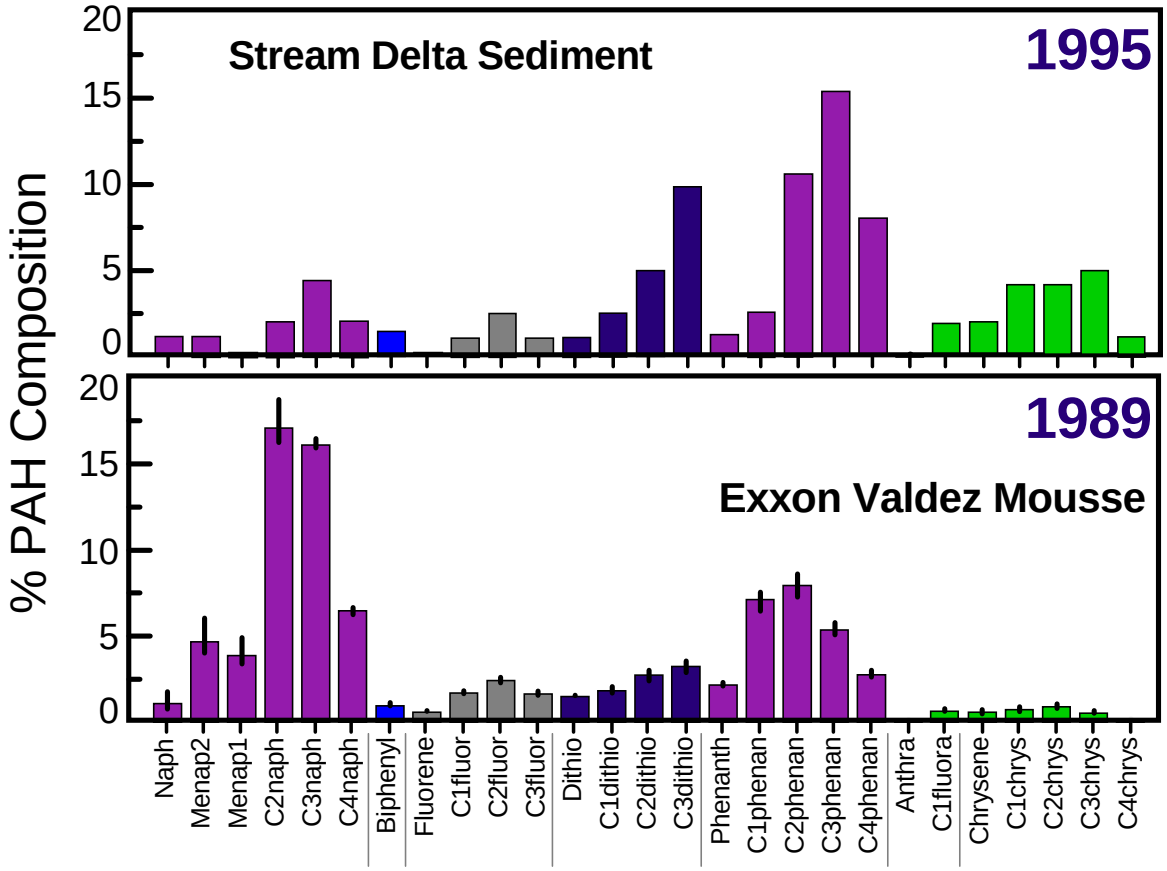

Figure 7. Relative concentration of polynuclear aromatic hydrocarbons (PAH) in a sediment sample from a stream delta (stream no. 16780) in Prince William Sound, Alaska, and in three samples (mean and range) of *Exxon Valdez* mousse (from Murphy et al., 1999, reprinted by permission). Names of PAH are abbreviations used in Short et al. (1996). The 1995 sample had a weathering index (Short and Heintz, 1997) of 5.3.

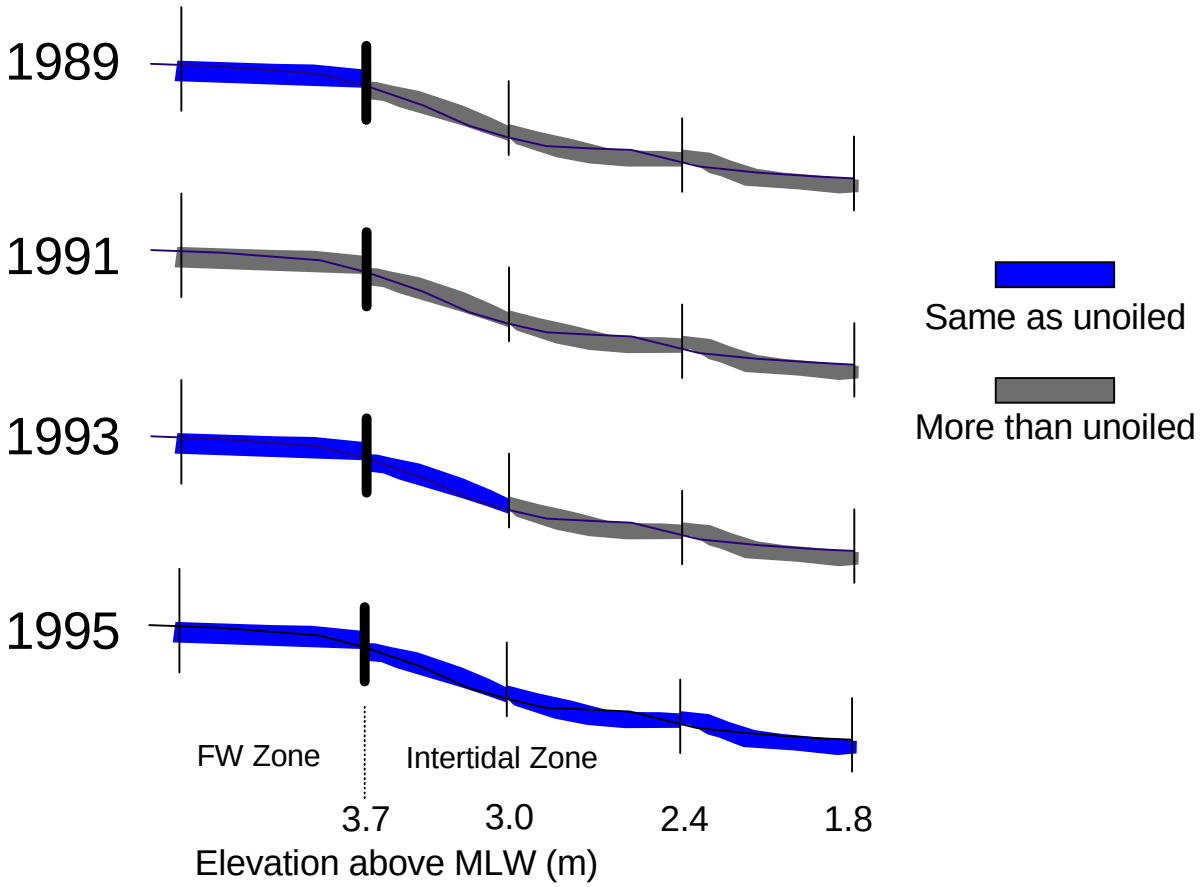

Figure 8. Elevated pink salmon embryo mortalities in oiled streams of PWS (from Bue et al., 1998). Unoiled streams (n=10) are compared to oiled streams (n=15) – differences are significant ($P<0.05$). Only the odd-year stock is presented; even year stocks were also significantly different in 1990 and 1992 (but not 1994).

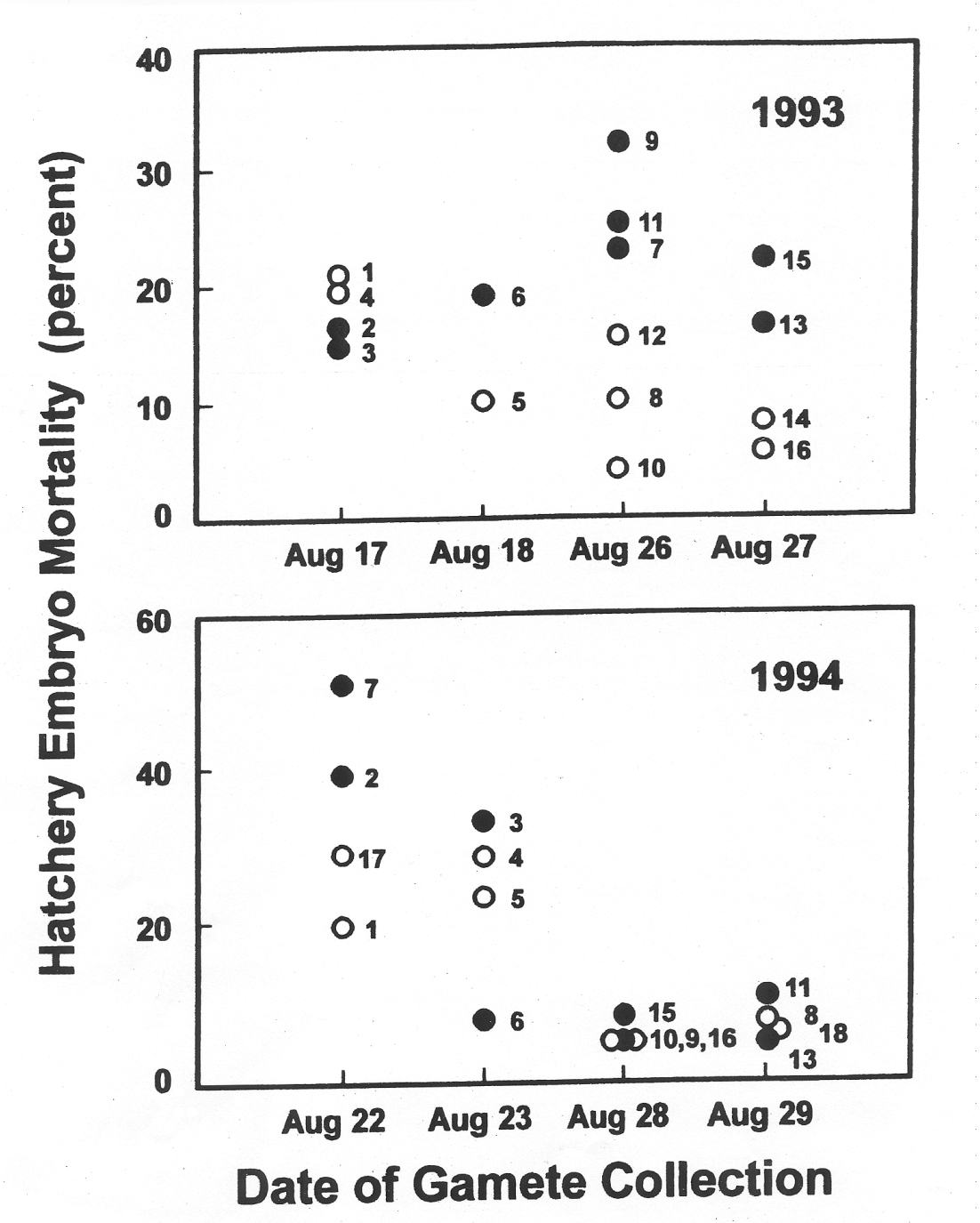

Figure 9. Mean mortality of pink salmon embryos observed in controlled incubation experiment in 1993 and 1994. Embryos were from oil-contaminated streams (solid circles) and reference streams (open circles); the number next to the circle identifies the stream location (from Bue et al., 1998, reprinted by permission).

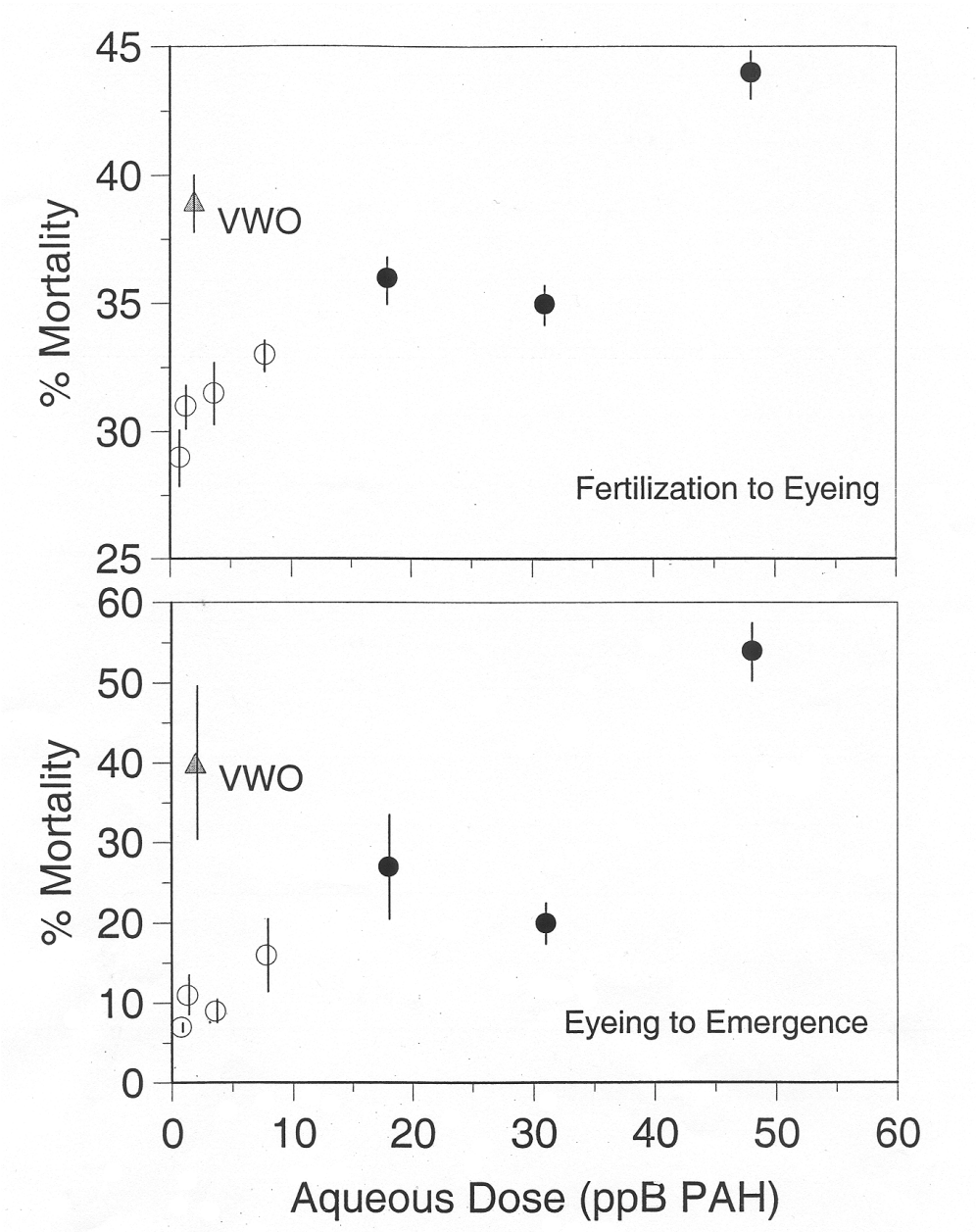

Figure 10. Mean mortalities rates (+- SE) for pink salmon embryos exposed to low aqueous PAH concentrations, including a dose with weathered oil (VWO). Mortality rates are expressed as the percentage of embryos dying between fertilization and eyeing or eying and emergence. Filled symbols are significantly greater than controls. Doses are initial aqueous concentrations, in ppB PAH. The compositions of PAH changes with weathering (from Heintz et al., 1999, reprinted by permission).

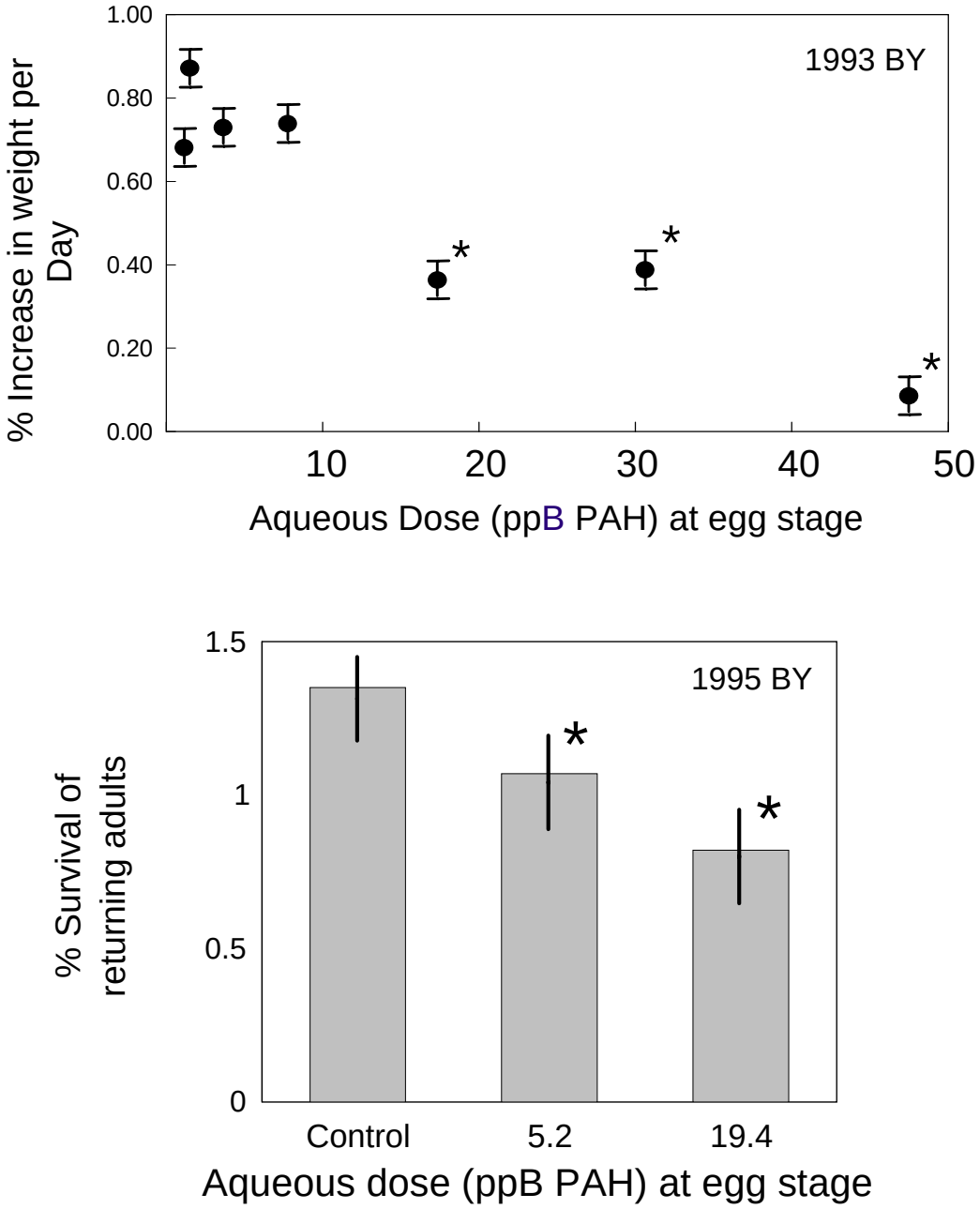

Figure 11. Delayed effects on fry growth of 1993 brood pink salmon fry 4-6 months after PAH exposures ended (top frame) compared to delayed effects on marine survivals of tagged fry (1995 brood) coming from exposed embryos; from Heintz et al. (in press). Approximately 70,000 tagged fry per dose group were released in the marine survival experiment. An asterisk indicates significant ($P<0.05$) difference from controls.